Review Article

Nanopolyphenols: Perspective on oxidative stress-induced diseases

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Abstract

Recently nanopolyphenols are gaining widespread interest in the drug discovery domain. Nanonization of polyphenols has greatly affected the therapeutic index owing to improvement in pharmacokinetic and biopharmaceutical obstacles linked with the use of natural polyphenols. They have been looking at an emerging paradigm for an array of disease symptoms. In this article, we have explored the therapeutic potential of nanopolyphenols in oxidative stress-induced diseases such as neurodegeneration, cancer, obesity, and diabetes. This article will present the current state of the art of various nanopolyphenols targeting oxidative stress-induced diseases. The advanced fabrication strategies presented for polyphenols including nanocrystal, mesoporous silica nanoparticles, nanoparticles, nanoliposome, gold nanoparticle, and nanosuspension are discussed. The information presented in light of recent *in vitro*, *in vivo*, and clinical evidence for nanoformulation and delivery of polyphenols may show a new dimension to future research in the realm of herbal therapy for oxidative stress-induced diseases. Significant information on the molecular mechanisms underlying linkages of oxidative stress with neurodegenerative diseases, cancer, obesity, and diabetes is discussed. Valuable information on dietary polyphenols in these diseases and their clinical data is presented. Based on different experimental evidence, the review findings support phenomenal therapeutic strategies for nanopolyphenolic fabrication with extended benefits and a condensed time frame. The status of clinical trials conducted on nanopolyphenols is presented. Although clinical trials conducted on nanopolyphenols for mentioned diseases are few, we have tried to present as much available clinical data in this article.

Keywords: Antioxidant, cancer, diabetes, nanopolyphenols, neurodegeneration, obesity, oxidative, stress

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INTRODUCTION

Nanotechnology is one of the pioneering technologies set to streamline the process of drug discovery in terms of speed, size, reliability, and automation which involves research and development at the atomic, molecular, and macromolecular levels. In the drug discovery and development sector, nanotechnology is geared toward the betterment of diagnostics like bioimaging and biosensor, formulation of drugs, and targeted drug delivery systems.[1,2] With the advent of nanotechnology, the number of nanomedicines is on a

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constant rise.[3] The unique chemical, physical, or biological features of these have offered new opportunities for research owing to the rise in the development of new delivery strategies, treatment modalities, approvals, and failures of drugs. Till now, a Large number of nanomedicines have been employed for cancer treatment and diagnostics and provide scope for other diseases too.[4] Recently, 3 nanomedicines-Patisiran/ONPATTRO, VYXEOS, and NBTXR3/Hensify got approval from FDA/EMA/CE mark. Patisiran/ONPATTRO, a lipid nanoparticle RNAi therapeutic approved by FDA (Food and Drug Administration) (2018) and EMA (European Medicines Agency) (2018) for Transthyretin (TTR)- mediated amyloidosis whereas VYXEOS is a liposomal formulation of cytarabine: daunorubicin which is approved from FDA,2017 and EMA (2018) for treatment of different leukemias. NBTXR3/Hensify is a hafnium oxide nanoparticle that got approval from CE Mark (European Conformity mark) (2019) for application to squamous cell carcinoma.[5] Since 2016, a clinical trial of 18 new nanoparticles has been registered. Out of these, 17 are used for cancer: 15 for treatment and 2 for imaging.[5]

Considering the potential and benefits of nanotechnology in drug discovery, we have explored the therapeutic potential of nanopolyphenols (NPs) in chronic disease management. Recently, a study supported the increased use of NPs over the last two decades, especially in the pharmaceutical and nutraceutical sector. As per the data, a huge number of NPs have been patented and commercialized. In the last three years (2019–2021), 97 NPs patents are published.[6] Therefore, this article presents information on advanced nano strategies for the fabrication of NPs that can be explored for the amelioration of oxidative stress-linked diseases like neurodegeneration, cancer, obesity, and diabetes. Moreover, we have focused on dietary polyphenols, as food/ diet rich in polyphenols are reported as vital in conditions like obesity, diabetes, high blood pressure,[7] cancer, and neurodegenerative disorders.[8] It is considered an antioxidant strategy that may show implications for various oxidative stress-induced symptoms. Thus as an approach to rectify pharmacokinetic and pharmacodynamic challenges that limit therapeutic potential and to enhance the therapeutic index of polyphenols or plant bioactive, information on advanced nano-strategies is discussed in this article for designing NPs that may give a new dimension to future herbal drug research.

The manuscript was prepared using data from clinical studies, in-vitro, in silico, and *in vivo* studies and review articles published till May 2022 in various journals, books, and e-resources. The search was limited to an article written in the English language. The keyword search included but was not limited to polyphenols, nanopolyphenols, oxidative stress, obesity, diabetes, neurodegeneration, cancer, natural molecules, medicinal plants, herbal medicine, nanotechnology, nanocarrier, drug delivery system, and clinical studies. The search engines used for data collection were Pubmed, Google Scholar, Science Direct, ResearchGate, arXiv, SemanticScholar, Clinical trials portal, etc.

OXIDATIVE STRESS AND INDUCED DISEASES

Oxidative stress is the condition produced due to disturbance of the balance between generation and buildup of Reactive Oxygen Species (ROS) and antioxidants that further lead to disturbance of redox signaling and molecular damage.[9] ROS production is the normal physiological phenomenon going on inside the cell due to aerobic metabolism and inflammatory responses.[10] Moreover, environmental factors like UV radiation, pollutants, chemicals, etc severely increase the ROS concentration enforcing the development of oxidative stress. Interestingly, ROS manifests the phenomenon of hormesis with one phase for redox functions at low concentration contributing to physiological functions including regulated cellular differentiation, tissue regeneration, and prevention of aging whereas on the other side in higher concentration produces oxidative stress targeting biological molecules like DNA, protein, lipids, enzymes and other small molecules adversely.[11] Oxidative stress has been found to play role in the initiation and development of a myriad of pathological conditions and diseases covering atherosclerosis, cancer, neurodegenerative disorders, hypertension, and diabetes.[12,13] How oxidative stress is involved in the induction/development of diseases is discussed in the next section.

Link between oxidative stress and neurodegenerative disease

Oxidative stress severely affects the central nervous system (CNS) and develops different neurodegenerative diseases due to high oxygen requirement, weak or dysfunctioning of antioxidant systems,[14] and occurrence of peroxidation-prone lipid cells in the brain.[15] Neurodegeneration represents multifactorial origin. Oxidative,[16] and nitrative stress is the central phenomenon during neuronal loss and neurodegeneration.[17] [Figure 1] shows the role of oxidative stress in the initiation and development of neurodegenerative diseases.[18-25]

Link between oxidative stress and cancer

The role of oxidative stress in the development of cancer multistage is revealed. [Figure 2] shows the involvement

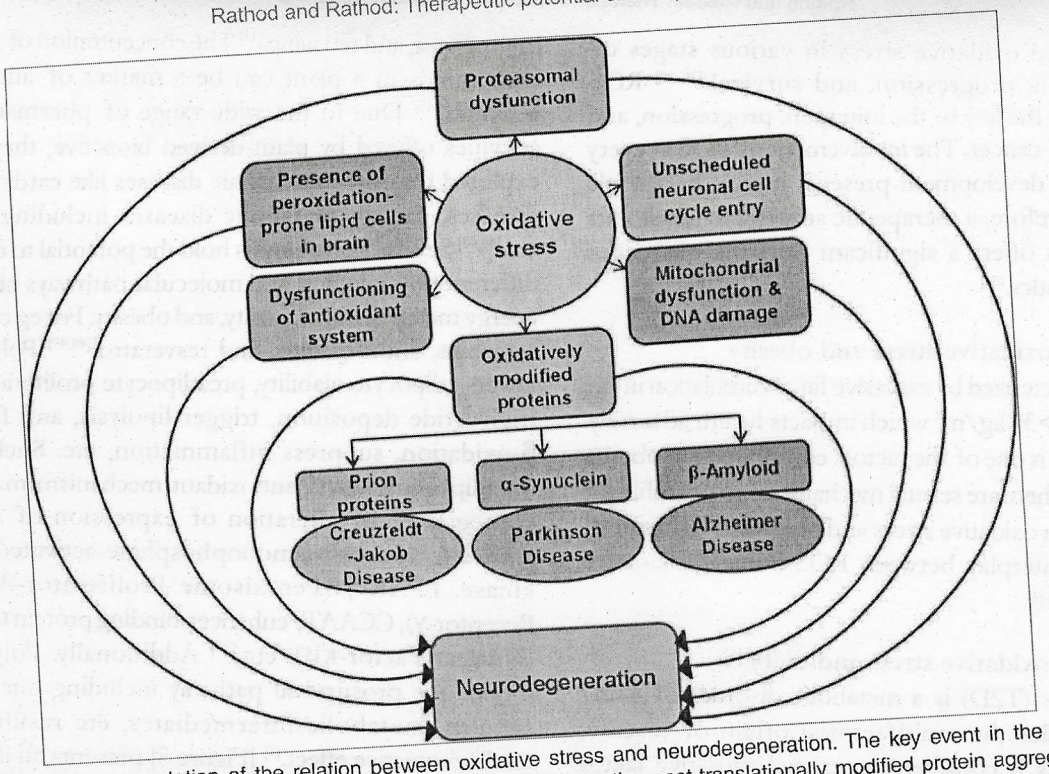

Figure 1: Schematic representation of the relation between oxidative stress and neurodegeneration. The key event in the pathogenesis of neurodegeneration is ROS-induced oxidative stress. Many oxidative and nitrative post-translationally modified protein aggregates have been considered a hallmark of several neurodegenerative diseases. Deposition of such aggregate induces a multitude of events like oxidative stress, proteasomal dysfunction, and mitochondrial dysfunction that continue up to neuronal cell death.[14-25]

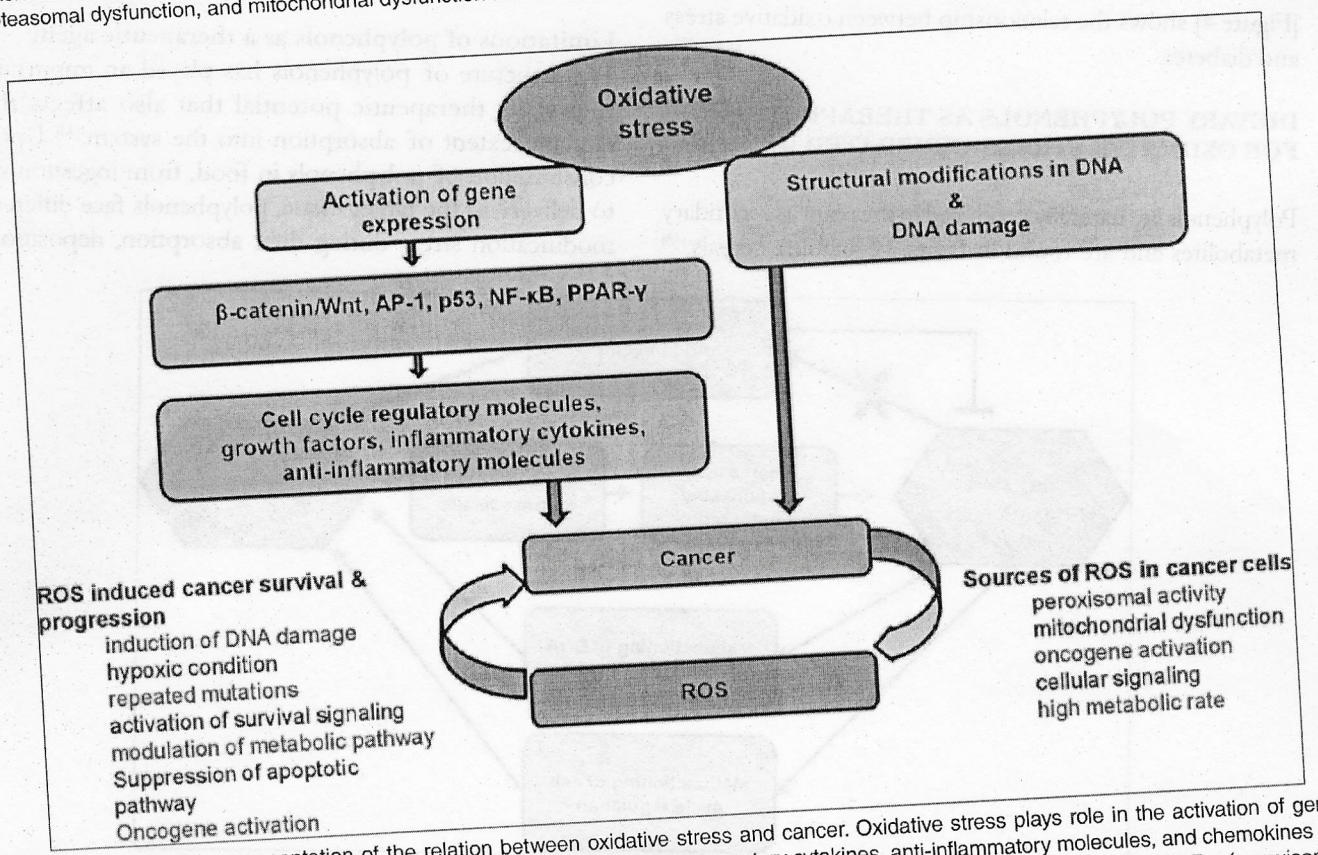

Figure 2: Schematic representation of the relation between oxidative stress and cancer. Oxidative stress plays role in the activation of gene expression for different cell cycle regulatory molecules, growth factors, inflammatory cytokines, anti-inflammatory molecules, and chemokines by activation of different transcription factors including β-catenin/Wnt, AP-1(Activator protein 1), p53, NF-κB (nuclear factor κ B), PPAR-γ (peroxisome proliferator-activated receptor-γ), etc affecting progression and survival of cancer.[26-29]

of ROS-induced oxidative stress in various stages of cancer initiation, progression, and survival.[26-29] ROS augmentation is the key to the initiation, progression, and development of cancer. The involvement of ROS at every step of disease development presents it as a therapeutic target.[30,31] Therefore, a therapeutic strategy to ameliorate oxidative stress offers a significant potential target for cancer therapeutics.[32]

Link between oxidative stress and obesity

Obesity is characterized by excessive fat accumulation in the body and BMI >30 kg/m^2 which impacts health adversely. Oxidative stress is one of the factors contributing to obesity development. There are several mechanisms responsible for the link between oxidative stress and obesity.[33-36] [Figure 3] indicates the interplay between ROS-induced oxidative stress and obesity.

Link between oxidative stress and diabetes

Type 2 diabetes (T2D) is a metabolic disorder of rising concern. Besides the multifactorial origin of disease, Oxidative stress is considered a crucial causative factor in the development of T2D, where ROS is involved in the initiation and development of insulin resistance.[37-42] [Figure 4] shows the relationship between oxidative stress and diabetes.

DIETARY POLYPHENOLS AS THERAPEUTICS FOR OXIDATIVE STRESS-GENERATED DISEASES

Polyphenols are naturally produced in the plant as secondary metabolites and are found in fruits, vegetables, cereals,[43] legumes, tea, and red wine.[44] The concentration of phenolic compounds in a plant can be a marker of antioxidant activities.[45] Due to the wide range of pharmacological activities offered by plant-derived bioactive, they can be explored for different chronic diseases like cardiovascular diseases, cancer, metabolic diseases including obesity, etc.[46,47] Several polyphenols hold the potential to modulate different physiological and molecular pathways underlying energy metabolism, adiposity, and obesity. For eg curcumin, catechins, anthocyanins, and resveratrol[48,49] Polyphenols lowers adipocyte viability, preadipocyte proliferation, and triglyceride deposition, trigger lipolysis, and fatty acid β-oxidation, suppress inflammation, etc. Such effects on adipogenesis and antioxidant mechanism may be the consequence of alteration of expression of signaling pathways adenosine-monophosphate-activated protein kinase, PPAR-γ (Peroxisome Proliferator-Activated Receptor-γ), CCAAT/enhancer-binding protein α, NF-κB (Nuclear Factor-κB), etc.[50] Additionally, Polyphenols induce the prosurvival pathway including microRNAs, sirtuins, metabolic intermediates, etc resulting in a cardioprotective effect.[7] [Figure 5] presents an illustrative diagram for dietary polyphenols as therapeutics for oxidative stress-generated diseases.

Limitations of polyphenols as a therapeutic agent

The structure of polyphenols has played an important role in its therapeutic potential that also affects the rate and extent of absorption into the system.[43] Upon consumption of polyphenols in food, from ingestion up to delivery to the target tissue, polyphenols face different modification stress during their absorption, deposition,

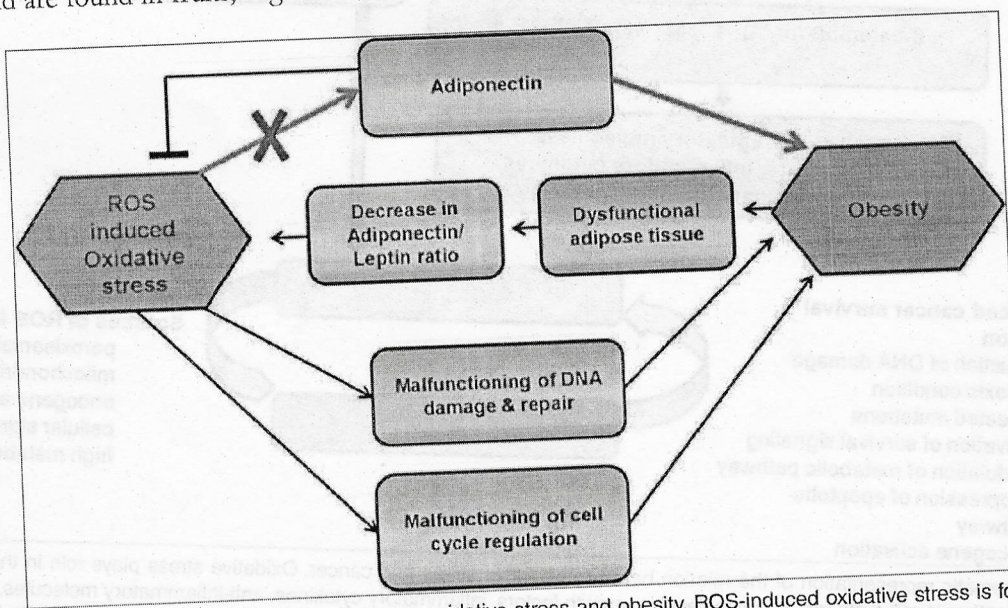

Figure 3: Schematic representation of the relation between oxidative stress and obesity. ROS-induced oxidative stress is found as the central player in the initiation and progression of obesity. Adiponectin is important for the amelioration of oxidative stress-induced damages. By inhibiting adiponectin production, ROS aggravates damage to the cell and nuclear processes thereby initiating the development of obesity.[34-36]

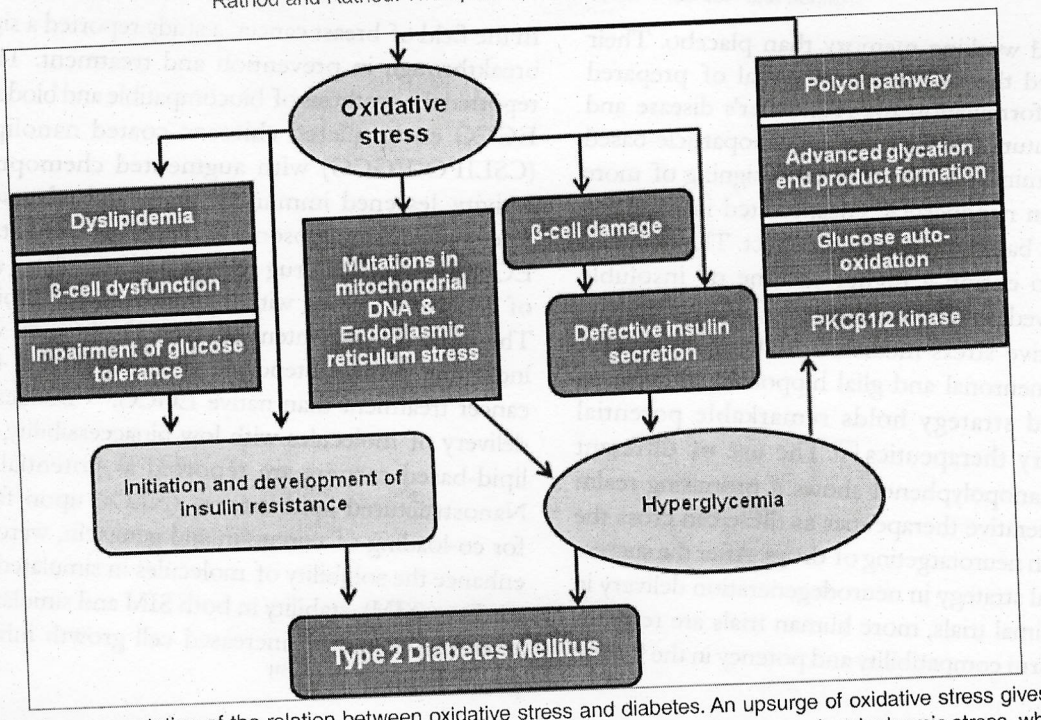

Figure 4: Schematic representation of the relation between oxidative stress and diabetes. An upsurge of oxidative stress gives rise to T2D by affecting different pathological events like dyslipidemia, β-cell damage, mitochondrial DNA damage, and endoplasmic stress, which gives rise to the production of T2D.[37-42]

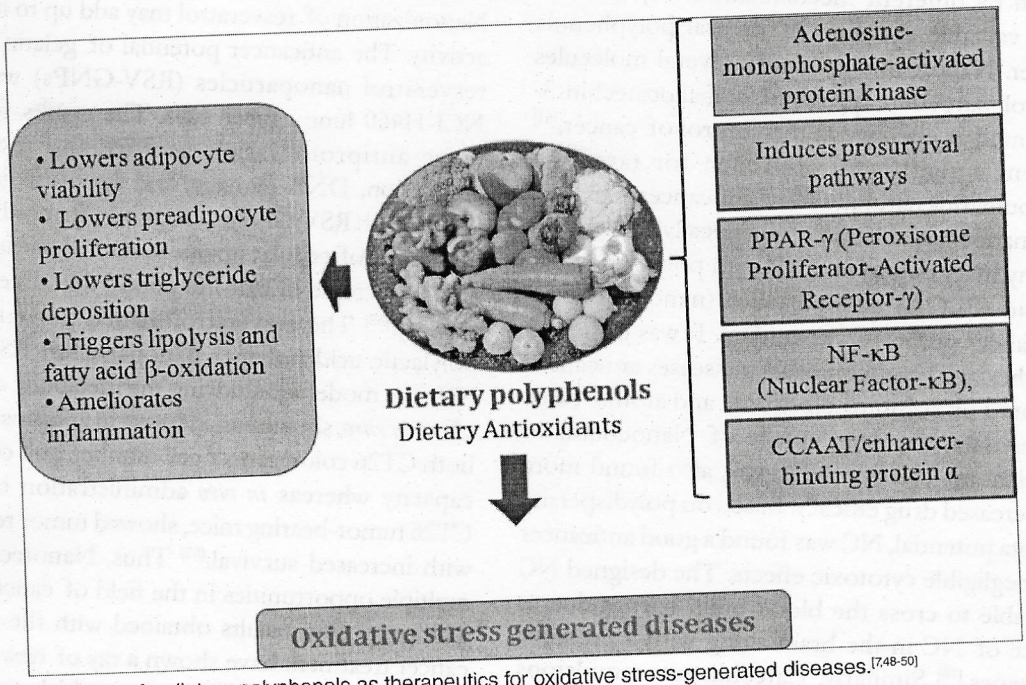

Figure 5: Illustrative diagram for dietary polyphenols as therapeutics for oxidative stress-generated diseases.[7,48-50]

metabolism, and excretion (ADME) which hence disturbs their bioactivity.[51] During the development of any drug, bioavailability is an important factor. It is the percentage of active drugs that finally come to systemic circulation or site of the action.[52] Invivo dietary antioxidants can only work as an antioxidant when they are present in an ample amount within bodily fluids and tissues.[53] The most challenging issue in the development of polyphenols as a therapeutic agent is the low bioavailability of polyphenols. Other factors like poor solubility, short half-life, low body absorption, *in vivo* stability, and tissue-specific delivery[54-56] limit their therapeutic potential. For example, the stability of EGCG in the intestine and blood is poor. It absorbs at a low rate and shows low bioavailability.[57] Similarly, the low bioavailability found with quercetin may be associated with its poor absorption, higher metabolism, or faster

cue memory and working memory than placebo. Their findings reported the significant potential of prepared Nanocurcumin formulation for Alzheimer's disease and supported the future development of nanoparticle-based drugs.[76] A preliminary work reported designing of more effective naringin nanoparticle encapsulated in modified PEG 3000 silica based on pegylation effect. The designed NP was able to entrap a higher volume of insoluble drugs and showed enhanced protection against amyloid β-linked oxidative stress-mediated Alzheimer's disease in primary rat neuronal and glial hippocampal cultures. The mentioned strategy holds remarkable potential as brain delivery therapeutics.[77] The use of different strategies for nanopolyphenol shows a promising realm for neurodegenerative therapeutics as these can cross the major obstacle in neurotargeting of drugs. After the success of this technical strategy in neurodegeneration delivery in in-vitro and animal trials, more human trials are required further to confirm compatibility and potency in the human system.

Nanopolyphenols for cancer

Nanonization by different mechanisms is reported to enhance the valuable activities of natural polyphenols against cancer. Nanoformulations of several molecules like resveratrol, curcumin, quercetin, epigallocatechin-3-gallate, etc found as effective in the control of cancer,[61] and represent a significant approach for targeting tumors.[55] Coumarins naturally show anticancer activities, the effect of nanoencapsulation of chemically synthesized 4-methyl-7-hydroxy coumarin (SC) with poly lactide-co-glycolide acid (PLGA) nanoparticles (nanocoumarin) on the anticancer property was studied. It was observed that the synthesized Nanocoumarin possesses anticancer activities. Based on scanning electronic and atomic force microscopies data, the drug uptake of Nanocoumarin into the melanoma cell line A375 was also found more represent increased drug efficacy. Based on polydispersity index and zeta potential, NC was found a good anticancer agent with negligible cytotoxic effects. The designed NC was found able to cross the blood-brain barrier due to the presence of NC in the brain along with a presence in other tissues.[78] Similarly, Catechin nanoemulsions produced from Oolong tea leaf waste were checked for anticancerous activity. The prepared nanoemulsion significantly inhibited prostate cancer cells DU-145 induced tumor in mice. Nanoemulsion prepared by adding lecithin, Tween 20, and water showed more stability and significant encapsulation efficiency of 83.4%. It was found efficient in lowering the size of mice tumors. The results obtained from the study supported further clinical trials of catechin nanoemulsions.[79]

In the field of breast cancer, a study reported a significant breakthrough in prevention and treatment. The study reported the synthesis of biocompatible and biodegradable EGCG encapsulated chitosan-coated nanoliposomes (CSLIPO-EGCG) with augmented chemopreventive activity, lessened immunogenicity, and adverse effects. The studied nanoliposome showed increased stability of EGCG, controlled drug release, and stimulates apoptosis of cancer cells along with inhibition of cell proliferation. The intracellular content of EGCG obtained was more indicating strong potency of CSLIPO-EGCG for breast cancer treatment than native EGCG.[80] Besides, for oral delivery of molecules with low bioaccessibility, coloaded lipid-based carriers are reported as potential vehicles. Nanostructured lipid carriers (NLCs) upon fabrication for co-loading of curcumin and genistein, were found to enhance the solubility of molecules in simulated intestinal medium (SIM), stability in both SIM and simulated gastric medium. Coloading increased cell growth inhibition of prostate cancer cells.[81]

Among various plant-derived molecules, Resveratrol is a molecule with vast pharmacological potential, Nanonization of resveratrol may add up to its therapeutic activity. The anticancer potential of gelatin encapsulated resveratrol nanoparticles (RSV-GNPs) was studied in NCI-H460 lung cancer cells. The results found showed more antiproliferation activity and increased ROS generation, DNA damage, and apoptosis in cancer cells treated with RSV-GNPs. It showed enhanced bioavailability, increment of cellular uptake sustained release of the drug, and an increase in half-life period than free RSV with no toxicity.[82] The resveratrol-loaded polyethylene glycol-polylactic acid polymer nanoparticles (RSV- PEG-PLA NP) in a model for studying the metabolic and anti-tumor effect *in vitro*, showed an increase in apoptosis, reduction in both CT26 colon cancer cell number, and colony-forming capacity whereas *in vivo* administration of RSV-NP to CT26 tumor-bearing mice, showed tumor regression along with increased survival.[83] Thus, Nanotechnology offers multiple opportunities in the field of cancer therapeutics. The promising results obtained with the use of NPs in cancer treatment have shown a ray of new hope and have opened new research dimensions which may help to untag the incurable term used for cancer.

Nanopolyphenols for obesity

In recent years, NPs have been explored for the antiobesity effect. In a study, Nanoemulsion oleoresin capsicum (NOC) upon administration to obese rats significantly lowered body weight and adipose tissue mass. Alteration in multiple gene expression, AMPK activation, and suppression of

elimination.[58] Certainly, research work focused to improve the stability of polyphenols and specific delivery of these molecules will be of great value.

NANOPOLYPHENOLS FOR OXIDATIVE STRESS-INDUCED DISEASES

Within a few years, many advances have been made in designing polyphenol on nano scaffolds to achieve enhanced bioavailability. The advantages are often accompanied by increased biocompatibility, nonimmunologic behavior, increased biodegradability, augmented strength of mucoadhesion,[59] and improves stability,[60] which leads to constant drug release thereby increasing the intracellular concentration of polyphenols. [Figure 6] represents different nanocarriers employed for the generation of nanopolyphenols able to efficiently attenuate ROS-induced oxidative stress and subsequent development of associated diseases. Different nanoformulations utilized for delivery of dietary polyphenols like solid lipid nanoparticles, polymeric nanoparticles, polymeric complex nanoparticles, liposomes, nanocrystals, gold nanoparticles, nanosuspensions, electrospun nanofibers, electro-sprayed nano-particles, and nano-spray dried particles and nano-caseins.[61,62]

Recently, for targeted drug delivery of natural products, Mesoporous silica nanoparticles (MSNs) of different morphology are gaining significant interest due to simple synthesis, significant chemical stability, biocompatibility, more drug loading capacity, and regulated drug release, tunable pore sizes. The safety aspect of MSNs has been supported by data obtained from various *in vivo* pre-clinical evaluations. These have been reported as an excellent nanocarrier for the stimuli-responsive release of drugs both internally and externally.[63] In a study to generate an integrated dual-responsive nanoplatform, mesoporous melanin-like polydopamine co-formulated with curcumin and silver nanoparticles was produced to study antibacterial

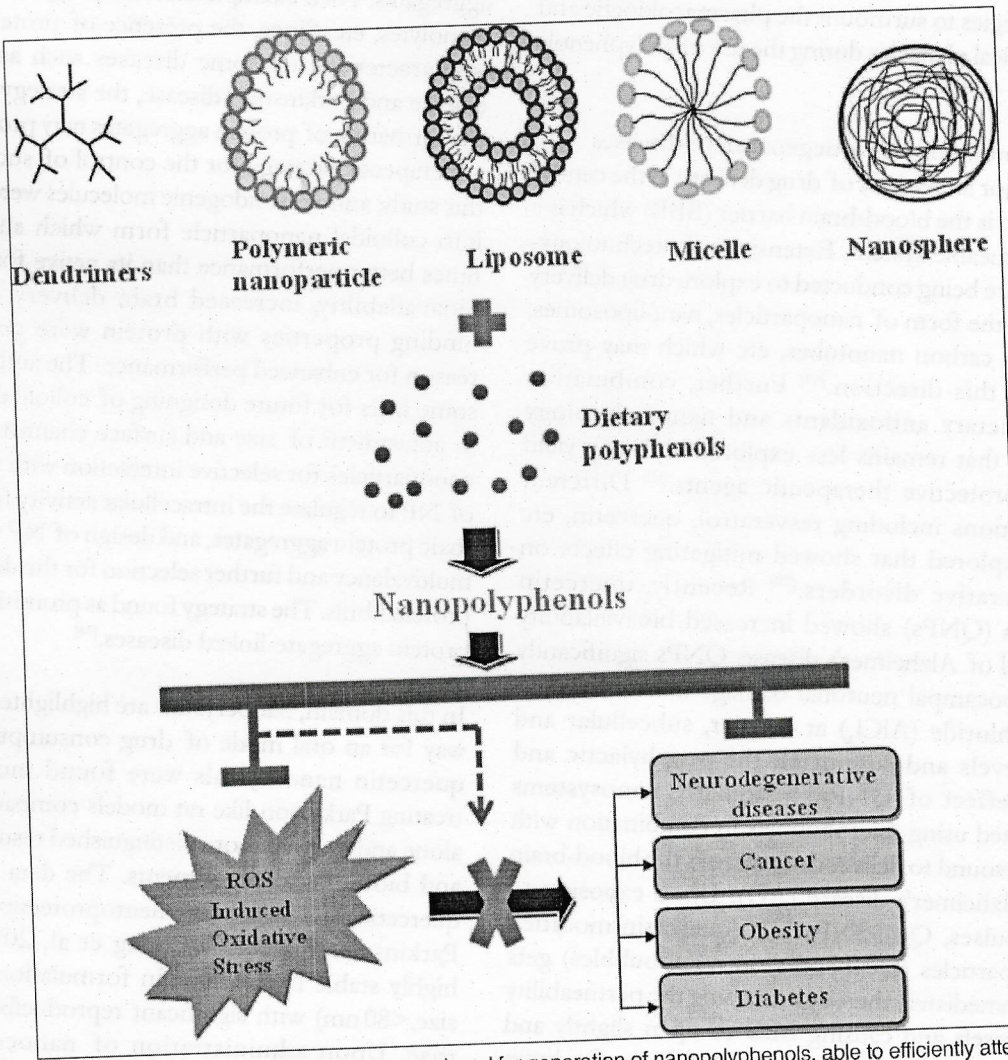

Figure 6: Schematic presentation of different nanocarrier employed for generation of nanopolyphenols, able to efficiently attenuate ROS induced oxidative stress and subsequent development of associated diseases

and antitumor properties. The generated CCM@SBA-15/PDA/Ag efficiently responded to pH and ROS stimuli to release the silver and curcumin. The nanoformulation showed enhanced bactericidal activity and chemotherapeutic ability against drug-resistant cancer cells. The study nicely presented the combinatorial design of natural molecules and silver nanoparticles to control infection and tumors in mice.[64] The cerium oxide nanoparticles synthesized from the proteome of *Justicia adhatoda* leaf show a significant increase in antioxidant activity and stability.[65] Clinical trials indicate that liposome formulations are found as more effective on pharmacological and pharmacokinetic parameters for the treatment of acute myeloid leukemia, ovarian cancer, breast cancer, hepatitis A, etc., and are one of the most potent, safe, and healthy nanoparticles generated so far.[66] Interestingly, Nanomedicine based on natural molecules like curcumin, camptothecin, paclitaxel, and resveratrol is already in the market and clinic with positive results.[67] Therefore, nanotechnology presents proficient strategies to surmount the pharmacokinetic and biopharmaceutical obstacles during the use of polyphenols [Figure 6].[68]

Nanopolyphenols for neurodegenerative diseases

One of the major limitations of drug delivery in the central nervous system is the blood-brain barrier (BBB) which is a selectively permeable system. Extensive nanotechnology-based studies are being conducted to explore drug delivery to the brain in the form of nanoparticles, nanoliposomes, nano micelles, carbon nanotubes, etc which may prove promising in this direction.[69] Further, combination therapy of dietary antioxidants and nanotechnology is a vast area that remains less explored and may yield novel neuroprotective therapeutic agents.[60] Different nanoformulations including resveratrol, quercetin, etc have been explored that showed mitigating effects on neurodegenerative disorders.[70] Recently, quercetin nanoparticles (QNPs) showed increased bioavailability in a rat model of Alzheimer's disease. QNPs significantly lowered hippocampal neuronal damage induced due to aluminum chloride ($AlCl_3$) at cellular, subcellular and molecular levels and supported the prophylactic and therapeutic effect of QNPs.[71] Similarly, nanosystems are constructed using microbubbles in combination with focused ultrasound to deliver drugs across the blood-brain barrier in Alzheimer's disease (AD). Upon exposure to ultrasonic pulses, Qc@SNPs-MB (quercetin-modified sulfur nanoparticles (Qc@SNPs) in microbubbles) gets destroyed immediately thereby increasing the permeability of blood vessels and causing BBB to open slightly and leading to the accumulation of Qc@SNPs in the brain parenchyma. This nanosystem efficiently overcomes the effects of endoplasmic reticulum stress and lowers oxidative stress, neuronal apoptosis, inflammation, and shielded nerve cells thus treating AD efficiently. This study reported a combination of Qc@SNPs-MB with ultrasound as a potential method for treating both neurodegenerative diseases and endoplasmic reticulum stress.[72]

It was observed that the treatment efficacy of neurodegenerative diseases especially Parkinson's disease, can be enhanced with the use of nanophytomedicine with a specific size of 1–100 nm. Nanosizing of phytobioactive molecules increases their stability and efficiency along with an increase in penetrating ability like ginsenosides (19.9 nm) showed an increase in bioavailability in the rat brain.[73] Recently, colloidal nanoparticles of anti-amyloidogenic small molecules are shown to inhibit the formation of protein aggregate, fragmentation of protein aggregates, and clearance of toxic protein aggregates. Antiamyloidogenic molecules are molecules inhibiting the formation of protein aggregates. Their example includes polyphenols, alkaloids, osmolytes, etc. Since, the presence of protein aggregates is characteristic of some diseases such as Alzheimer's disease and Parkinson's disease, the strategy of inhibiting the formation of protein aggregates may prove valuable as a therapeutic strategy for the control of such diseases. In this study, anti-amyloidogenic molecules were transformed into colloidal nanoparticle form which showed 100000 times better performance than its native form. Improved bioavailability, increased brain delivery, and modular binding properties with protein were reported as the reason for enhanced performance. The authors suggested some facts for future designing of colloidal nanoparticles as adjustment of size and surface chemistry of colloidal nanoparticles for selective interaction with protein, design of NP to regulate the intracellular activity for clearance of toxic protein aggregates, and design of NP with controlled multivalency and further selection for the disintegration of protein fibrils. The strategy found as promising for targeting protein aggregate-linked diseases.[74]

In this domain, nanocrystals are highlighted as an efficient way for an oral mode of drug consumption. In a study, quercetin nanocrystals were found more efficient in treating Parkinson-like rat models compared to quercetin alone and showed more distinguished results in behavioral and biochemical experiments. The data supports nano-quercetin as a significant neuroprotection candidate for Parkinson's disease.[75] Cheng et al, 2013 developed a highly stable nanocurcumin formulation (mean particle size,<80 nm) with significant reproducibility and storage ease. Upon administration of nanocurcumin in the Alzheimer's disease model Tg2576 mice showed good

glycerol-3-phosphate dehydrogenase in white adipose tissue might be the reason behind the antiobesity effect of NOC.[84]

A clinical trial with nano curcumin (NC) on overweight/obese NAFLD patients reported significant findings. NC showed improved glucose indices and decreased HbA1c, TG, TC, LDL, and inflammatory markers.[85] Since overweight and increased oxidative stress are correlated, The antioxidant potential of cerium oxide nanoparticles (nanoceria) was evaluated as a therapeutic strategy for the management of obesity. In 3T3-L1 pre-adipocytes, nanoceria by suppressing transcription of adipogenic genes and hampering triglyceride accumulation drove modulation of adipogenic pathway while in Wistar rats, nanoceria efficiently lowers body weight, levels of insulin, glucose triglyceride, and leptin in plasma. The results supported the anti-adipogenic properties of cerium oxide nanoparticles and reported the promising potential of nanoceria in obesity treatment.[86] To perform targeted delivery of resveratrol nanoparticles to adipose stromal stem cells (ASC), a target peptide was incorporated on the nanoparticle surface; ASC targeted nanoparticles (ATnano). The synthesized ATnano showed enhanced intracellular concentration in primary stromal vascular fraction. It showed increased deposition of resveratrol in white adipose tissue (WAT). The results highlighted the strategy as a significant breakthrough in obesity treatment.[87] Additionally, the antiobesity strategies based on nanotechnology include the betterment of intestinal health, lowering of energy intake, targeting cell abnormalities, maintaining redox balance and elimination of free lipoprotein from blood,[88] antiangiogenesis, the transformation of WAT to brown adipose tissues, and photothermal lipolysis of WATs,[89] which reflects the importance of nano strategies in disease management.

Nanopolyphenols for diabetes

Nanopolyphenols are gaining importance in diabetes research. In this direction, quercetin nanorods (QCNr) showed efficient antioxidant and antidiabetic activities in alloxan-induced diabetic mice. This system efficiently improves lipid peroxidation and protein carbonylation, augmentation of antioxidant enzymes like SOD (Superoxide dismutase), and catalase along with improvement in liver and kidney activities.[90] Despite various techniques, the High-Pressure Homogenization method employed for the preparation of drug-loaded nanosuspensions is more efficient and productive than other methods. Based on this method, preparation of berberine nanosuspension (Ber-NS) composed of Ber and D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) and administration to streptozotocin-induced diabetic C57BL/6 mice, effectively ameliorates T2D by lowering blood glucose and improvement of lipid metabolism. A significant decrease in body weight and good hypoglycemic and total cholesterol levels was reported.[91] Further, Pluronic nano micelles loaded with curcumin (CURnp) were evaluated for antidiabetic potential using pancreatic tissues. CURnp triggers *Pdx-1* and *NKx6.1* gene expression and maintains redox balance thereby improving streptozotocin-induced β-cell damage by activating insulin gene expression. An increase of 40% insulin-positive cells proved the antidiabetic potential of CUR-loaded pluronic nanomicelles.[92]

Interestingly, a combination therapy involving coenzyme Q10 and curcumin both encapsulated separately in poly(D, L-lactic-co-glycolic acid)-based nanoparticles upon administration to streptozotocin-induced diabetic rats showed a decrease in the levels of plasma triglycerides and total cholesterol with a concomitant increase of the level of HDL-cholesterol. The data support the potential of nanoparticulate formulations of coenzyme Q10 and curcumin in diabetes management.[93] In a study, ferulic acid encapsulated chitosan nanoparticles (FANPs) were synthesized by the ionotropic gelation process. Upon administration to streptozotocin (STZ) induced diabetic Wistar albino rats, improved hyperglycemic activities with regulated lipid profile. The result marked the potential of encapsulated FANPs in diabetes management and highlighted the strategy useful to avoid the derived hurdles observed during the use of synthetic drugs.[94]

Yucel et al, 2018 developed new RSV-loaded nanoliposomal formulations and incubated them with pancreatic β TC cell-induced with glucose and streptozotocin. The synthesized liposomal formulation showed prolonged antioxidant activity. A significant lowering of glucose levels along with a synchronous increase in insulin levels was observed. The data reported resveratrol nanoliposomes as a novel approach to target diabetes mellitus.[95] Additionally, Resveratrol nanoemulsion (nano-Resv) lowers the level of serum glucose and improves the level of serum insulin in STZ-induced diabetic rats.[96] In a study, individual effects of curcumin nanoparticles (Curc-NP), zinc oxide nanoparticles (ZnO-NP), and curcumin-zinc oxide composite nanoparticles (Curc-ZnO-NP) were evaluated on streptozotocin-induced diabetic rats. Lowering of blood glucose, increased insulin levels, and maintenance of GLUT-2 and glucokinase gene were observed. Besides, Curc-ZnO-NP out of three nanoparticles was found to show the most potent anti-diabetic activities based on histopathological findings.[97] In a double-blind randomized clinical trial, the effect of nano-curcumin(NC) was studied on HbA1C, fasting blood glucose (FBG), and lipid profile

in 70 diabetic patients. NC was found to show an HbA1c lowering effect on type-2 diabetes along with a significant lowering of fasting blood glucose, triglyceride, and BMI.[98] In addition to nanopolyphenols, Nanotechnology has helped in diabetes research in diagnostics, monitoring, and treatment.[99]

Undoubtedly, Nanonization has tremendously augmented the therapeutic properties of polyphenols and subsequent improvement in the therapeutic index. Still, there is a shortage of clinical trials on NPs in the field of oxidative stress-generated diseases. In the present scenario, as per data search, capsaicin nanoparticles and nano curcumin was only found to get evaluated under clinical trial. Capsaicin nanoparticles were evaluated on patients with painful diabetic neuropathy whereas 5 clinical trials have been performed with nano curcumin on different disease conditions- Multiple sclerosis (completed), Ankylosing spondylitis (completed), recurrent aphthous ulcer and recurrent aphthous stomatitis (completed), Prostate cancer (active), Metabolic syndrome (completed).[100] Upsurge in the number and frequency of clinical trials are sincerely required to further validate the data. Perhaps, the challenge faced while the clinical translation of nanomedicine is appropriate designing in a way that it remains stable during systemic circulation and shows the controlled release of drugs at the right site. Immunotoxicological studies are required before clinical translation of nanomedicine as nanocarrier is found to modulate immune responses and is left in the body.[67] The use of proper standards and correct controls, and reporting of details during research may resolve difficulties encountered while the development of nanotechnology-based therapeutics.[4]

CONCLUSION

Nanotechnological advances made in the area of drug discovery and development in terms of nanomaterial-based targeted drug delivery have revolutionized the research. Based on a wide array of experimental evidence including in-vitro, animal trials, and clinical trials favors NPs in treatment strategies for many disease symptoms of oxidative stress origin. Different nano-strategies explored so far have proven the augmentation of pharmacological activities due to improvement not only in the specificity and selectivity of drug delivery to target tissue but in the bioavailability, stability, and intracellular concentration of polyphenols to drive the optimal activity. Despite the observed benefits, more well-controlled clinical studies are of the essence which is sincerely needed to further validate the data.

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Nil

Conflicts of interest
There are no conflicts of interest.

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Effect Of Non-Surgical Periodontal Therapy On Levels Of Salivary MMP- 8 In Chronic Periodontitis Patients: Systematic Review

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Introduction

Chronic periodontitis is a condition that results in the loss of tooth-supporting connective tissue and alveolar bone and, if untreated, is a major cause of tooth loss in adults. [1] It is a prevalent condition, affecting 47.2% of the adult US population aged 30 years or older. [2] According to the Centre for Disease Control and Prevention and American Academy of Periodontology case definitions, the prevalence of moderate and severe periodontitis are estimated as 30.0% and 8.5%, respectively, among US adults. [1]

Periodontitis, when viewed from a histopathological perspective, is characterized by a complex interaction between periodontal bacteria and host inflammatory response which results in the release of pro-inflammatory cytokines which leads to the destruction of supporting periodontal structure and the alveolar bone.[3] One of the important host factors responsible for collagen destruction and extracellular matrix destruction is Matrix Metal loproteinases (MMP). Many MMP are studied over the years and the important ones are MMP -1, 8, 9, 13, and 14. [3]

Chronic Periodontitis presents in various forms and it's challenging for the clinicians to manage the varying extent and severity the disease presents. Treatment of Chronic periodontitis can range from Scaling and Root Planning (SRP) to SRP with adjunctive treatments to surgical interventions.[1] The options chosen however can vary and depend on the degree of Chronic Periodontitis. The effect of treatment of chronic Periodontitis is reflected in the improvement of clinical parameters and the Histological parameters.

When a Literature search was carried out we came across a few systematic reviews [4, 5] which showed that chronic periodontitis patients had increased levels of MMP8 levels. Thus we decided to go a step further and decided to see if non-surgical therapy brings about a reduction in the MMP8 Levels.

On literature search, it was noted that along with SRP procedure along with other adjunctive therapies were available which reduced the MMP 8 levels.

Thus the PICOS was designed accordingly.

Population- aged 18- to 58-year-old having chronic periodontitis.

Intervention – Scaling and root planning/ Scaling and root planning along with Adjuvant therapy.

Control- subjects with clinically healthy periodontium.

Outcome - Change in salivary MMP- 8 levels in chronic periodontitis patients.

Study design – Randomized control trial

Material and methods

Literature Search/Data Base Search

Extensive literature search was carried out using the following electronic databases, amending the search strategies Trial Register of the Cochrane Oral Health Group (to 18 December 2015),Cochrane Central Register of Controlled Trials (CENTRAL) (2015, Issue 11), MEDLINE via Ovid (1946 to 18 December 2015), EMBASE via Ovid (1980 to 18 December 2015), HINARI, Science Direct, Google Scholar, Ebsco host and Wiley.

Also, US National Institutes of Health Trials Register (http:// clinicaltrials.gov) (to 18 December 2015) and Clinical Trial Registry-India were used as search engines. Free ones have been downloaded directly by the URLs generated from the database. The restricted group has been downloaded by the institutional access of the KAU library.

Search Strategy

The Mesh terms used were "Matrix Metal loproteinases 8" "Chronic Periodontitis" "Non-Surgical Periodontal therapy" "Scaling and Root Planning". The Boolean operators used were "AND" and "OR".

We placed no restrictions on the language and no time frame restriction of the publication was used for searching the electronic databases. Where only a relevant title without a listed abstract was available, a full copy of the article was assessed for inclusion. The references of included articles were checked for additional studies suitable for inclusion. The articles were assessed by two independent authors. Reference lists were checked to identify any other articles that might provide information relevant to the research question.

67 records were obtained from various search engines, of which after assessment, 27 duplicates were removed, of which 40 were screened and 25 full-text articles which were not available as full text were excluded. 15 full articles were assessed for eligibility. 04 studies were excluded as they don't meet the inclusion criteria. Finally, 11 articles were included for the synthesis of the systematic review. **(Details in the PRISMA flow chart).**

Inclusion & Exclusion Criteria

The inclusion and exclusion criteria as mentioned in Table 1.

Inclusion Criteria
• Patients aged 18-58 years • Studies which studied MMP-8 levels by either Saliva, GCF samples • Study having a control group • Randomised Controlled Trails
Exclusion Criteria • Study having Patients above 58 years • Study having Medically compromised patients like Diabetes, Hypertension, Cardiac History • Studies that assessed MMP 8 levels in Aggressive Periodontitis • Studies that assessed MMP 8 levels in Juvenile Periodontitis

Article review

Two independent authors reviewed the articles. Only articles that complied with the inclusion criteria were reviewed further. Full copies of articles were reviewed independently by two reviewers (add reviewers initial) for compliance with the exclusion criteria. Those articles that assessed MMP 8 levels by any of the sample fluid be it Saliva, GCF or blood were included. Also all the studies which used ELISA method or any other method for MMP8 levels were included.

Data Extraction

A systematic data extraction sheet was constructed. The details included were Author, Year of publication, Country where study was carried out, Sample size, Fluid which was collected, Study group, Control group, Methodology, Duration of study and results of the study.

Results

Data Summary of Included study

Totally 11 articles were included in the study. 1 study used Plasma as sample to assess the MMP8 levels, saliva was used as medium in 3 studies and majority of studies i.e. 7 studies used Gingival Crevicular fluid (GCF).

Data of Excluded Studies

Based on the exclusion criteria 4 studies were excluded because of the following reasons. 2 Studies were done on Aggressive Periodontitis patients, 1 Study was done on Juvenile Periodontitis patients and 1 study was done in systemically compromised (Diabetic) Patients.

Discussion

MMP-8 and its Fluid Sources

MMP s are proteolytic enzymes that belong to the zinc protease superfamily involved in the physiological degradation of extracellular matrix proteins and basement membranes, and they are often categorized into several groups. A significant source of MMP-8 (neutrophil-type MMP-8) in humans is degranulation triggered by neutrophils, but MMP-8 (mesenchymal cell-type MMP-8) is

additionally de-nova expressed and secreted in small amounts by non-PMN-lineage cells like epithelial cells, smooth muscle cells, fibroblasts, macrophages, and endothelial cells.[5]

MMP-8 which is one of the important members of the MMPs family and belongs to the collagenous group exhibits a novel ability to decompose type I and III collagen which are found within the periodontal ligament. Therefore, it will be hypothesized that MMP-8 acts as a biomarker in periodontitis. [6]

The main source of oral salivary collagenase is PMNs that enter the mouth through the gingival sulcus. GCF levels of MMPs in patients with periodontitis were also studied in terms of their diagnostic and prognostic values.[7]

7 studies out of the 11 studies included used GCF because the sample to assess the MMP8 levels. 9 studies used the ELISA test to analyse the fluid and one study by Kinane DF[8] used monoclonal antibodies and the other study by Pozo[9] used Western blotting.

MMP8 and Scaling and Root Planning (SRP)

Smiley C J et al.[1] in their article discusses strong evidence-based recommendations that SRP helps in reducing the MMP 8 levels yet the Clinical Parameters of the diseases such as Bleeding on Probing, Probing depth, loss of attachment reduced over time. The author also recommends that along with SRP adjuvant therapies may be combined to assist bring the disease in check. [1]

In the current systematic review, 11 studies are included within which 4 studies compare the extent of reduction of MMP-8 levels after scaling and root planning only. Other 7 studies together with SRP other adjuvant therapy was used. 3 studies - Sub microbial Dose of Doxycycline was used as an adjuvant; 1 study - hyperbaric oxygen therapy was used; 1 study - Probiotic Mouthwash was used and last 1 study- used Ozonized Oil as an adjuvant.

The 4 studies which showed that SRP reduced MMP-8 levels were Kinane DF et al.[8], Pozo P et al.[9], Marcaccini AM et al [10], Sexton W M et al.[11] All of them compared MMP-8 levels after the Scaling and Root Planning (SRP) procedure between healthy patients and patients with periodontitis.

Adjuvants used along with SRP

Three studies Choi DH et al, [12]Tuter G et al, [13]Emingil G et al.[14] used sub antimicrobial dose of Doxy cycline as an adjuvant together with SRP procedure. In all three studies, it was observed that MMP 8 levels reduced much further when used along with adjuvants than SRP alone. Doxy cycline is one of the tetracycline derivatives that are known for its effectiveness in antibiotic applications. It is widely used because of its effectiveness in suppressing periodontopathic microorganism and has been used widely as an adjunct to periodontal therapeutic agents.[12]

Kanagaraj SS et al [15] used probiotic product merchandise was a combination of BIFILAC-lozenges, which had the following composition contains Lactobacillus sporogenous 100 Million, Streptococcus faecalis T-110 JP C 6 0 million, Clostridium butyrium TO- A4 million, and Bacillus mesenteric TO-A JPC 2 million. [15]

Nardi NP [16] used Ozonized olive oil as an adjuvant and both groups showed statistically significant differences. Ozone has analgesic and anti-inflammatory actions and is thought to act by a different

mechanism of action: The suggested mechanisms are (1) Decreased production of inflammatory products and (2) Inactivation of metabolic products that mediate pain by oxidation and by improving the vascular supply that ends up in increased oxygen availability to tissue and better rate of toxic product elimination. It further improves tissue regeneration and quickens wound healing. Moreover, ozone is a negatively charged ion, thus it rebalances the acidic environment in infection. [16]

Soranta N.P [17] used Hyperbaric Oxygen therapy (HBOT) together with traditional SRP was used. It was seen that the reduction in MMP 8 levels was statistically significant when SRP was combined with HBOT than SRP used alone. Hyperbaric oxygen therapy is a therapy method where one inhales 100% pure oxygen in a high-pressure room of more than 1 ATA (Absolute Atmosphere). Chen T and his colleague's (2002) study showed that HBOT increased oxygen distribution at the bottom of the periodontal pocket, it could inhibit the growth of anaerobic bacteria and also allowed ischemic tissue to receive adequate oxygen intake for rapid recovery of cellular metabolism.[18]

Hendiani I et.al [19] used mangos teen peel extract which is an herbal extract. It was administered within the pocket depth and examined after 15 days. This was one such study that didn't show a statistically significant difference compared to SRP alone.

Conclusion

The reduction in GCF MMP-8 levels following therapy indicates that MMP-8 is one molecule that may eventually prove useful as an indicator of current disease status and possibly as a predictor of future disease. The fact that simple Scaling and Root Planning (SRP) can reduce an important marker of inflammation is incredible. The use of simple adjuvants further proves to be a better option than SRP alone. Today there are many adjuvants available that can provide synergistic to SRP in reduction of periodontal inflammation. However, more clinical trials need to be carried out which helps us decide which of the adjuvants are better.

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Review Article | Published: 13 September 2022

Anti-plasmodial and mosquitocidal potential of metallic nanoparticles: a perspective

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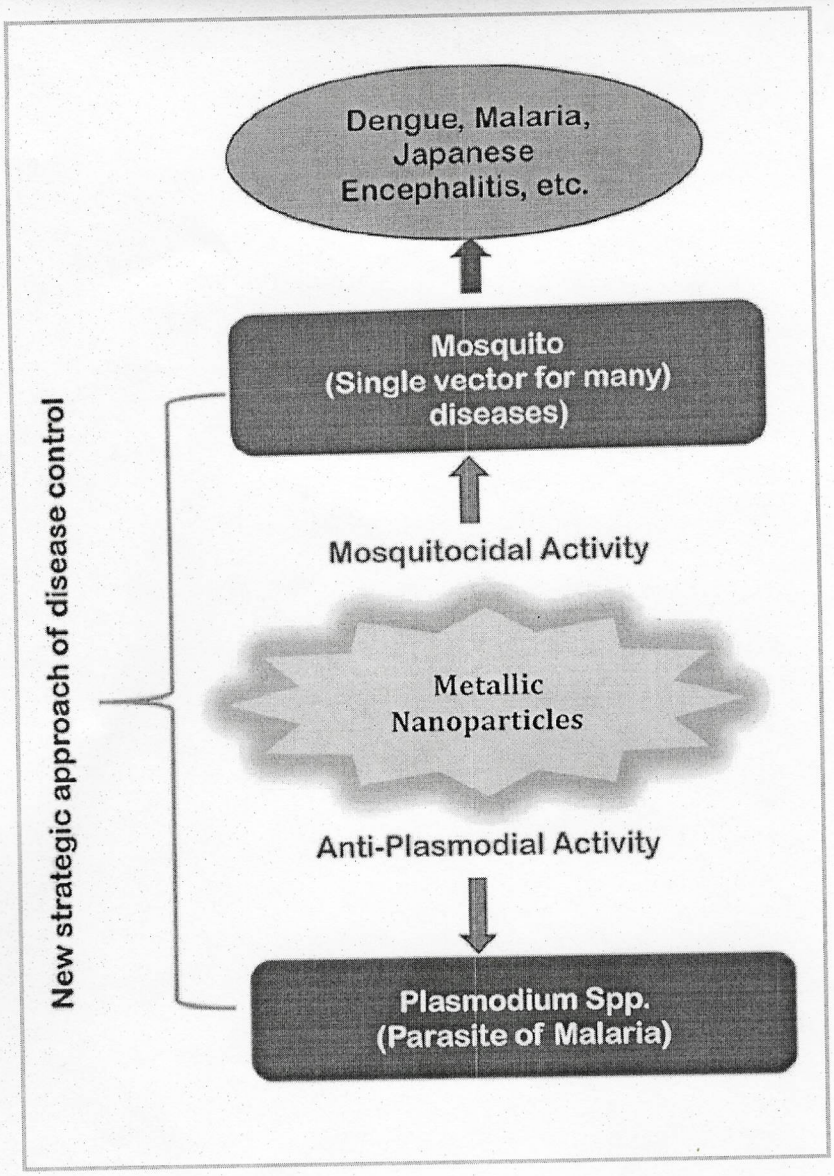
Abstract

The incidence of malaria, a mosquito-borne infectious disease exists throughout the tropical and subtropical regions. Various factors restrict the effective control of malaria including their increasingly resistance to existing anti-malarial drugs. New strategies for malaria management are needed at both the fronts for the search of new anti-malarial agent and control of vector anopheles in order to restrict the transmission of malaria. Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, filariasis, dengue, Japanese

encephalitis, etc., causing millions of deaths every year. Although many strategies including chemicals and phytochemicals has been well explored for parasite and vector control, in recent years, metal nanoparticles are gaining attention as potential material in this domain. This review has summarized, the present status of some metallic nanoparticles as anti-plasmodial and mosquitocidal activities in perspective of malaria management.

Graphical abstract

Fig. Metallic nanoparticles potential with Anti-plasmodial and Mosquitocidal activity



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ORIGINAL ARTICLE

Study of chloroquine susceptibility potential of plants using *pseudomonas aeruginosa* as in vitro model

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Abstract

Chloroquine (CQ) is mainly known for antimalarial activity but due to lower sensitivity, it has not been well explored in the microbial disease treatment. In the present investigation, we attempted to enhance the CQ sensitivity in *Pseudomonas aeruginosa*. Presence of efflux pump is well demonstrated in bacterial system which plays an important role in drug sensitivity and resistance in bacteria and also serves other functions. Taking the advantage of presence of efflux pump in *Pseudomonas aeruginosa*, we made an attempt to sensitize the *Pseudomonas aeruginosa* with various plant extracts and phytochemicals for the development of CQ sensitivity. Ten rationally selected plant extracts were screened for the development of chloroquine sensitivity in *P. aeruginosa*. The chloroquine susceptibility assay was demonstrated by combining CQ and verapamil (a known efflux pump inhibitor) as a standard in an in vitro assay system. Results were quite encouraging as methanolic extracts of *Syzygium aromaticum, Zingiber officinale* and *Curcuma longa* were able to enhance chloroquine sensitivity in *P. aeruginosa* by increasing the zone of inhibition in well-defined assay system. These plant extracts were finally analysed for the presence of various phytochemicals. The *Syzygium aromaticum* extract showed the presence of phytochemicals, such as quinones, phenol, triterpenoid, saponins, tannins, alkaloids and flavonoids. On the other hand, the methanolic extract of *Zingiber officinale* and *Curcuma longa* showed the presence of saponins, tannins, alkaloids and flavonoids in the extract. Towards the identification of active principle of selected plant extract for CQ sensitivity enhancement, thin-layer chromatography was performed and various phytocomponent bands were isolated. Flavonoid (R_f 0.44) in *Syzygium aromaticum,* alkaloid (R_f 0.43) in *Zingiber officinale* and phenol (R_f 0.62) in *Curcuma longa* were found responsible for the enhancement of CQ susceptibility in *P. aeruginosa*. This interesting finding confirmed the concept that a prior course or combination of plant extracts or phytochemicals with chloroquine can be effective against *P. aeruginosa*. Present investigation successfully presented the proof of concept for the enhancement of chloroquine sensitivity in bacterial system by modulating an efflux pump. Concept can be explored for repurposing chloroquine for new applications.

Keywords Chloroquine sensitivity · Antibacterial activity · *Pseudomonas aeruginosa* · Plant efflux pump inhibitors · Plant extracts · Phytocomponents

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Introduction

Development of antibiotic resistance in bacteria is a worldwide health problem that needs prime research attention to discover and develop a new drug or repurpose existing drug for new applications. Antibiotics, a class of drugs that once considered the lifeline against several bacterial infections are now under the threat of development of drug resistance in most pathogenic microbes (Shriram et al. 2018). During development of drug resistance, a decrease in the sensitivity of the drug is the primary stage. Several mechanisms have been reported for development of drug resistance, such as drug inactivation, target alteration, decreased permeability

and increase efflux pumps. Among these mechanisms, an increase in efflux pump expression which results in drug extrusion is the important mechanism associated with multi drug resistance (Sun et al. 2014 and Mohanty et al. 2021). In this line, sensitivity enhancement of drugs using efflux pump inhibitors or ion channel blockers presents a significant strategy for control of drug resistance. In general, efflux pumps are transport proteins-based systems in bacteria that are mainly involved in extrusion of substrates from the cellular interior to the external environment. These substrates are drugs often antibiotics, that communicate the efflux pump-expressing bacteria with antibiotic-resistant phenotype (Piddock 2006a). The characteristic of poly-substrate specificity of efflux pumps makes them to expel a broad range of antibiotics. They are also known to activate the acquisition of additional resistance mechanism by lowering the intracellular drug concentration. Moreover, various pieces of evidence have suggested that in bacteria, efflux pumps have physiological functions and their expression is tightly regulated in response to various environmental and physiological signals (Sun et al. 2014). Beside the drug resistance development, the role of efflux pumps in bacteria includes colonization, bile tolerance in case of enteric bacteria, enhancement in virulence, survival in the host (Piddock 2006b) and biofilm secretion. The drug efflux is considered one of the important mechanisms for development of antimicrobial resistance in biofilm structures in several bacteria including *P. aeruginosa* (Soto 2013). According to the list of bacterial species published by the World Health Organization in 2017, *Pseudomonas aeruginosa* categorized as one of the priority bacteria for which new antibiotics are urgently needed (WHO 2017). It rapidly develops resistance to multiple classes of antibiotics. In this opportunistic pathogen, antibiotic efflux is one of the most predominant mechanisms in which the administered drug is proficiently resisted (Housseini et al. 2018). Hence, there is an utmost need of therapeutic strategies for lowering resistance or increasing sensitivity of drugs towards *P. aeruginosa*.

Since developing a new drug is a time taking process and the drugs that are currently in the practice are the results of years of research and development. Therefore, repurposing of an old drug with strategic use for new activity or improved activity can be an effective approach.

Chloroquine a known antimalarial drug that has revolutionized the treatment of malaria, belongs to a large series of 4-aminoquinolines (Coatney 1963). It has been also explored in the treatment of rheumatoid arthritis, systemic lupus erythematosus, dermatological diseases, various types of cancer and in viral infections including use in COVID-19. Due to multiple mechanisms of action, such as pH-dependent inhibition of functioning and signalling of cell organelles, immunomodulatory actions, inhibition of autophagy and interference with receptor binding, it has emerged as a choice of molecule among researchers for studying various mechanism-based applications (Schrezenmeier and Dörner 2020; Pelt et al. 2018; Varisli et al. 2019). In this direction, the act of alkalinisation of acid vesicles in cells infected by intracellular bacteria and fungi indicates it is a good choice to treat bacterial and fungal infections (Hackstadt and Williams 1981). As *P. aeruginosa*, is an opportunistic pathogen known for its ability to rapidly develop the resistance against various antibiotics, the discovery of alternative drugs and treatments are needed. Thus, the current study has made an effort to evaluate the antibacterial potential of the known antimalarial drug CQ against *P. aeruginosa*.

Considering the current scenario, drug sensitivity enhancement using efflux pump inhibitors may act as a faster solution. Since plant-derived molecules are also reported for efflux pump inhibitory potential (Stavri et al. 2007), the present study explored the potential of plant as source of efflux pump modulator for the CQ susceptibility development in *P. aeruginosa*.

Materials and methods

Biological materials

Plants selected in this study were *Azadirachta indica* (leaf), *Menthe longifolia* (leaf), *Zingiber officinale* (rhizome), *Coriandrum sativum* (leaf), *Allium sativum* (bulbs), *Syzygium aromaticum* (bud), Curcuma longa (rhizome), *Piper nigrum* (fruit), *Phyllanthus emblica* (fruit), *Moringa oleifera* (leaf). These plants were purchased from the local market of Raigad district, Maharashtra India. All the plants were authenticated from Agharkar Research Institute, Pune, Maharashtra, India. The bacterial strain of *P aeruginosa* used in this study was authenticated by biochemical methods carried out at Lal Pathology Laboratory, Mumbai. 16S rRNA sequencing identification of the bacterial strain was carried out at Agharkar Research Institute, Pune, Maharashtra, India.

Chemicals

Chloroquine diphosphate salt (98.5–101.0% EP)- Sigma, Verapamil hydrochloride (≥99%)- Sigma Aldrich, Methanol (LR)- SD Fine Chemical Limited, Dimethyl sulfoxide (LR) 99%—SD Fine Chemical Limited, Mueller–Hinton agar—HiMedia, N-Hexane 99% (LR)- Chemico, Diethyl ether (99%)-Merck, Chloroform (extra-pure)- Sisco Research Laboratories, Sulphuric acid(concentrated)-SD Fine Chemical Limited, Ferric chloride (98%)-Merck, Sodium hydroxide (97%)- Merck, hydrochloric acid (35–38%)- SD Fine Chemical Limited were used in this study.

General experimental procedures

The study was designed using different methods for evaluation and isolation of active principle with CQ susceptibility enhancement potential (Fig. 1). The study was started with evaluation of antibacterial activity of CQ and VRP against *P. aeruginosa* using the agar well diffusion method. The development of CQ susceptibility was determined by combining CQ and VRP against *P. aeruginosa* using agar well diffusion method. Further, methanolic extract of ten plants were screened for their development of CQ sensitivity potential in *P. aeruginosa*. Based on the sensitivity potential, three plants were selected for further studies. The plant extracts were qualitatively investigated for the presence of various phytochemicals and chemical fingerprints were analysed using thin-layer chromatography (TLC). To identify the active principle, the bands were scratched from TLC plate and phytochemicals were isolated and identified. Various isolated phytochemicals were screened for CQ susceptibility development potential in *P. aeruginosa*.

Preparation of plant extracts

Rationally selected plants were used for preparation of extract (Stavri et al. 2007). Plants were first washed thoroughly under running tap water followed by sterile distilled water and then air dried. The dried plants were coarsely powdered and subjected to methanolic extract preparation. The extraction was carried out using methanol (1gm in 10 ml) and kept overnight at room temperature. The extracts were then filtered with Whatman filter paper No. 5 and the filtrate was subjected to methanol evaporation. After the complete evaporation of methanol, the dried extracts were suspended in Dimethyl sulfoxide (DMSO) and stored at the temperature of −20°C for further study.

Antibacterial activity of CQ against *P. aeruginosa*

The antimicrobial activity of CQ against *P. aeruginosa* was investigated by agar well diffusion method (Seasotiya and Dalal 2014; Performance Standards for Antimicrobial Disc Susceptibility Tests, Dahiya and Purkayastha 2012). The sensitivity of CQ against *P. aeruginosa* was determined using various concentrations of CQ from 0.9 to 500 µg/ml. Culture with 103 CFU/ml was used in this study. The

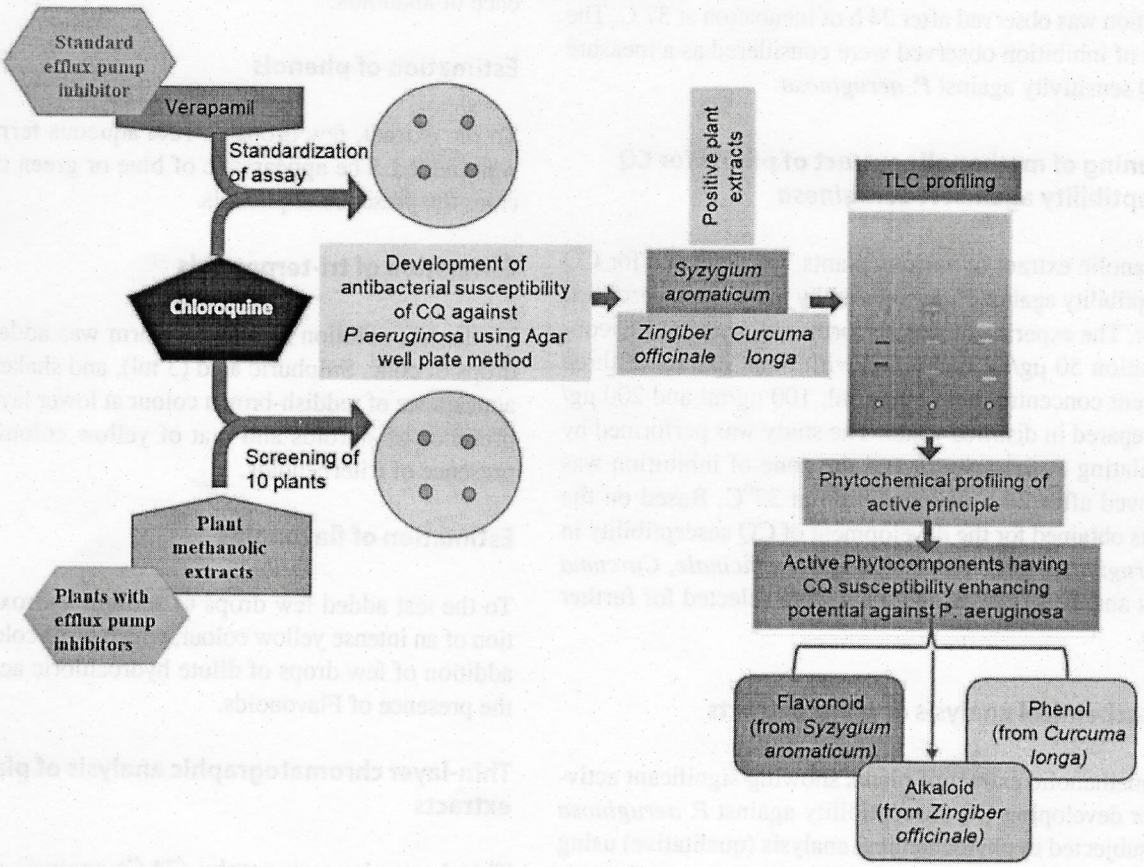

Fig. 1 Graphical presentation of strategy adapted for evaluation of plant extracts for CQ susceptibility development in *P. aeruginosa*

culture was gently swabbed on the sterile Mueller–Hinton agar plates and using a sterile borer, wells were prepared (5 mm diameter). The wells were labelled according to the CQ concentrations used in the experiment. The various concentrations of CQ prepared were added to the respectively labelled wells. The plates were incubated at 37°C for 24 h. The antibacterial activity of CQ and VRP was determined based on the zone of inhibition formed.

Antibacterial susceptibility of CQ using VRP against *P. aeruginosa*

The antibacterial susceptibility assay of chloroquine was performed using agar well diffusion method (Dahiya and Purkayastha 2012; Palaksha et al. 2013; Bhattacharjee et al. 2016). VRP is a known efflux pump modulator and calcium channel blocker (Sharma et al. 2019) was used as a standard. The non-inhibitory concentrations of CQ against *P. aeruginosa* were selected for developing antibacterial susceptibility. The CQ susceptibility assay was designed by keeping CQ concentration constant i.e., 50 µg/ml combined with various concentration of VRP i.e. 50 µg/ml, 100 µg/ml and 200 µg/ml. The concentrations used for CQ control was 50 µg/ml and for VRP was 200 µg/ml. The experiment was performed by inoculating *P. aeruginosa* and the zone of inhibition was observed after 24 h of incubation at 37°C. The zones of inhibition observed were considered as a measure of CQ sensitivity against *P. aeruginosa*.

Screening of methanolic extract of plants for CQ susceptibility against *P. aeruginosa*

Methanolic extract of various plants was screened for CQ susceptibility against *P. aeruginosa* by the method described earlier. The experiment was performed by keeping CQ concentration 50 µg/ml combined with plant extract at three different concentrations- 50 µg/ml, 100 µg/ml and 200 µg/ml prepared in distilled water. The study was performed by inoculating *P. aeruginosa* and the zone of inhibition was observed after 24 h of incubation at 37^{0}C. Based on the results obtained for the development of CQ susceptibility in *P. aeruginosa*, three plants - *Zingiber officinale*, *Curcuma longa* and *Syzygium aromaticum* were selected for further study.

Phytochemical analysis of plant extracts

The methanolic extract of plants showing significant activity for developing CQ susceptibility against *P. aeruginosa* was subjected to phytochemical analysis (qualitative) using various biochemical tests. The Biochemical tests for quinones, saponins tannins, alkaloids, phenols, triterpenoids and flavonoids were carried out (Prabhavathi et al. 2016; Shah and Seth 2010).

Estimation of quinones

To 1 ml of the extract, 1 ml of concentrated sulphuric acid was added. Formation of red colour shows the presence of Quinones.

Estimation of saponin

To 1 ml of the extract, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.

Estimation of tannins

To the extract, ferric chloride was added, formation of a dark blue or greenish black colour showed the presence of tannins.

Estimation of alkaloids

To the extract, 2 ml of Wagner's reagent was added, the formation of a reddish-brown precipitate indicates the presence of alkaloids.

Estimation of phenols

To the extract, few drops of 10% aqueous ferric chloride were added. The appearance of blue or green colour indicates the presence of phenols.

Estimation of tri-terpenoids

To the test solution 2 ml chloroform was added with few drops of conc. Sulphuric acid (3 ml), and shaken well. The appearance of reddish-brown colour at lower layer indicates presence of steroids and that of yellow colour shows the presence of triterpenoids.

Estimation of flavonoids

To the test added few drops of sodium hydroxide, formation of an intense yellow colour, which turns colourless after addition of few drops of dilute hydrochloric acid indicates the presence of Flavonoids.

Thin-layer chromatographic analysis of plants extracts

Thin-layer chromatography (TLC) analysis was carried out for the methanolic extracts of plant- *Zingiber officinale*

to establish the chloroquine susceptibility development using VRP, a known calcium channel blocker (Sharma et al. 2019) and efflux pump inhibitor-based assay model. Considering the efflux pump inhibitory characteristic, of VRP, it was the choice as a standard for development of CQ susceptibility assay in this study. Here, the test organism *P. aeruginosa* in which the efflux pumps are well reported and it is known for development of resistance against variety of drugs. The combination of modulator along with drug could be the essential factor for combination therapy, but pharmacokinetic data of modulator and drug should complement each other for successful therapeutic combination systems (Lomovskaya and Bostian 2006). Considering these complex factors, use of modulators from plant origin can be the promising approach with low toxicity advantage. The plant extracts are considered as the good source for drug development. Many plants are known for enhancing the drug sensitivity in bacteria through efflux pump inhibition (Stavri et al. 2007). Considering the current situation of multiple drug resistance development and the requirement of a long-time window for new drug development, our study attempts to develop antibacterial susceptibility of CQ against *P. aeruginosa* using plant materials. Using the CQ sensitivity development assay, ten plants were screened against *P. aeruginosa.*

Antibacterial activity of CQ against *P. aeruginosa*

The antimicrobial activity of CQ was investigated by exposing various concentrations of CQ (0.9–500 µg/ml) to *P. aeruginosa* using the agar well plate method. The zone of inhibition was not observed at the highest used concentration of CQ (500 µg/ml) in this assay (Fig. 2). The result indicates that *P. aeruginosa* does not show sensitivity against CQ up to 500 µg/ml concentration.

Antibacterial activity of VRP against *P. aeruginosa*

Prior to establishment of drug sensitivity development assay, a preliminary antibacterial activity of VRP against *P. aeruginosa* was carried out. On exposing various concentrations of VRP to *P. aeruginosa*, the zone of inhibition was not observed up to 500 µg/ml concentration. This indicates that *P. aeruginosa* does not show sensitivity for VRP up to 500 µg/ml concentration.

Antibacterial susceptibility of CQ using VRP against *P. aeruginosa*

The development of an antibacterial susceptibility assay of CQ was established using VRP by combining it with CQ to determine its potential to develop CQ susceptibility against *P. aeruginosa*. The assay was established by taking non-inhibitory concentrations of both test drug CQ and VRP. Out of three combinations of CQ: VRP screened (1:1, 1:2 and 1:4), the development of a zone of inhibition with 11.8 ± 0.11 mm diameter was observed in the test set having 1:4 ratio, whereas in other two sets with 1:1 and 1:2 ratios, no zone of inhibition was observed (Fig. 3). No zones of inhibition were observed in both the control sets of CQ (50 µg/ml) and VRP (200 µg/ml).

The formation of zone of inhibition indicates the development of antibacterial susceptibility of CQ against *P. aeruginosa* when combined with VRP in a 1:4 ratio. The study by Pieterman et al. (2018), reported the use

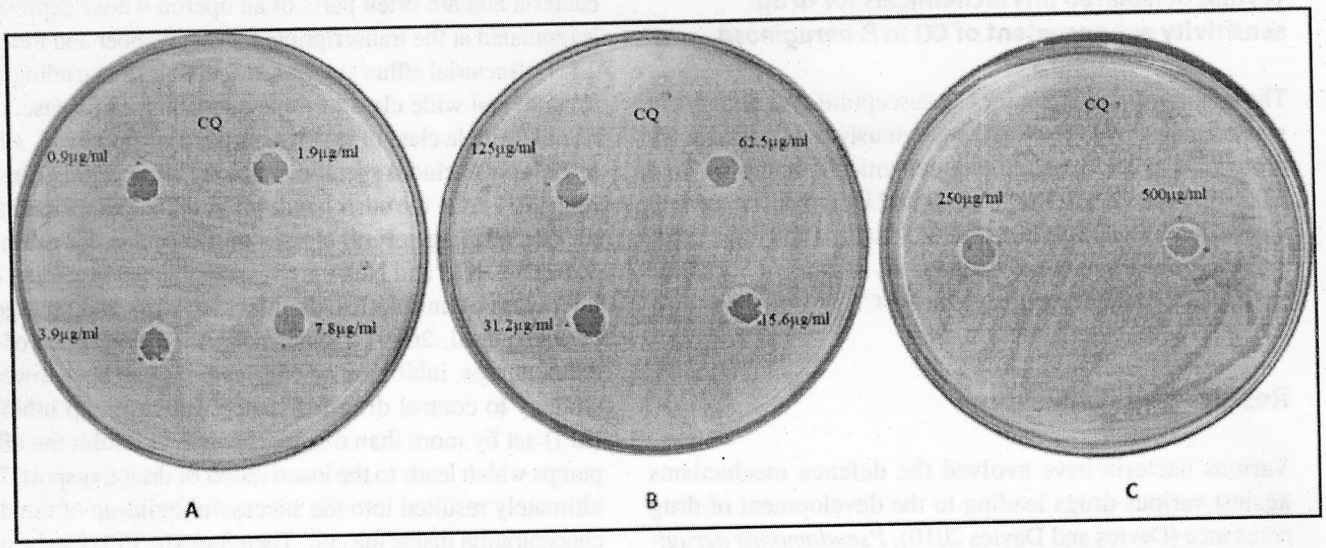

Fig. 2 Antibacterial activity of CQ against *P. aeruginosa*. The plates A, B and C represent a series of different concentrations (from 0.9 to 500 µg/ml) of CQ exposed to *P. aeruginosa*. The zone of inhibition was not observed in any of the plate which indicates that CQ does not inhibit the growth of *P. aeruginosa* up to 500 µg/ml concentration

(rhizome), Curcuma longa (rhizome) and *Syzygium aromaticum (bud).* Aluminium TLC silica gel 60 F_{254} sheet (Merck Life Science) were used as stationary phase for the analysis. For *Zingiber officinale extract*, n-hexane: diethyl ether in the ratio 4:6 (Rai et al. 2006) was chosen as mobile phase whereas, for *Curcuma longa* (Kushwaha et al. 2021) and *Syzygium aromaticum* (Hemalatha et al. 2016), chloroform: methanol in the ratio 9:1 was chosen as mobile phase. The TLC silica sheets were loaded with plant methanolic extracts on lower end and then dried. After the drying process, the sheets were placed in the saturated solvent chamber till the solvent front reached to 70% distance of the stationary phase. Following this, the plates were taken out of the chamber, dried and visualised under an ultraviolet cabinet (Runali Scientific, Mankhurd, India) followed by derivatization of bands developed with iodine vapours. The Retention factor (Rf) of developed bands was determined.

Isolation of various bands from TLC plate

To isolate phytocomponents from selected plant extracts for drug sensitivity enhancement of CQ in *P. aeruginosa*, the bands developed on the TLC sheets were scratched out, dissolved separately in methanol and centrifuged at 3000 rpm for 10 min. The supernatant was collected in new cleaned pre-weighed tubes and subjected to methanol evaporation. The pre and post weight of dried tubes were used to estimate the weight of band isolated. Stock solution (50 µg/ml) of isolated bands were prepared with dimethyl sulfoxide and used further for screening of CQ susceptibility development in *P. aeruginosa* followed by qualitative phytochemical characterization using biochemical analysis described earlier.

Testing of isolated phytochemicals for drug sensitivity enhancement of CQ in *P. aeruginosa*

The qualitative screening for CQ susceptibility development was estimated by following the previously established assay protocol. CQ and isolated phytochemical principles from TLC were used in a 1:1 ratio (50 µg/ml individual concentration) against *P. aeruginosa*. The CQ susceptibility against *P. aeruginosa* was measured on the basis of zone of inhibition formed after 24 h of incubation at 37°C.

Results and discussion

Various bacteria have evolved the defence mechanisms against various drugs leading to the development of drug resistance (Davies and Davies 2010). *Pseudomonas aeruginosa* is one such bacterium considered amongst six superbugs that have developed various mechanisms of resistance against multiple drugs that cause serious illness and death in humans with chronic and immunosuppressive conditions. It is a known opportunistic pathogen and considered as a model bacterium for virulence and bacterial social traits studies (Diggle and Whiteley 2020). The scenario of development of drug resistance constrained to repurpose the existing drugs for treatments other than their conventional ones. CQ and hydroxychloroquine have been used as secondary drugs to treat a variety of chronic diseases as they have anti-inflammatory properties and mechanism of action through drug accumulation in lysosome (Mauthe et al. 2018). Use of CQ has gained interest in treatment of infectious diseases (Rolain et al. 2007). Considering the sensitivity of the drug, CQ can effectively act as an antibacterial agent against *E. coli* and *Proteus vulgaris* (Jagadeesh et al. 2014). CQ and its analogues are considered as promising agents to use against bacterial and fungal infections due to their interesting mechanism of alkalinisation of acid vesicles that inhibits the bacterial and fungal growth (Rolain et al. 2007). CQ is repurposed for various treatments other than the malaria. In the present study, an attempt was made to develop an antibacterial susceptibility of CQ against *P. aeruginosa* at low concentration. A study related to combination therapy (Adegbolagun et al. 2008) showed the effect of combination of ciprofloxacin hydrochloride with chloroquine phosphate on selective strains of *P. aeruginosa* and *Klebsiella pneumoniae* isolates, which indicated a positive effect of combination therapy. The development of drug resistance in bacteria is a complex and multifunctional mechanism (Gandhi et al. 2013). Efflux pumps are known to be involved in the drug resistance development process, unlike most other determinants of resistance. As the efflux pumps are the assembly of transport proteins of bacteria and the genes coding for these transporters are found in both susceptible as well as resistant bacteria and are often parts of an operon whose expression is regulated at the transcriptional level (Webber and Piddock 2003). Bacterial efflux systems are capable of extruding both specific and wide class of molecules. In specific case, only one or a single class of drug is extruded such as TetA, which selectively excludes specific antibiotic tetracycline (Sharma et al. 2017). On the other hand, MDR efflux pumps are capable of extruding several classes of molecules, for example, MexAB-OprM and NorA are responsible for extruding distinct class of antibiotics, disinfectant, dyes and detergents (Sharma et al. 2019). Considering the importance of the efflux pumps, inhibition of efflux pumps can be a powerful strategy to control drug resistance. Efflux pump inhibitor (EPI) act by more than one mechanism to inhibit the efflux pumps which leads to the inactivation of drug transport. This ultimately resulted into the successful build-up of the drug concentration inside the cell. Therefore, the EPIs can be used along with the drug as an adjunct to enhance the sensitivity of the drug against the efflux pump-expressing bacteria (Sharma et al. 2019). In the present study, attempt was made

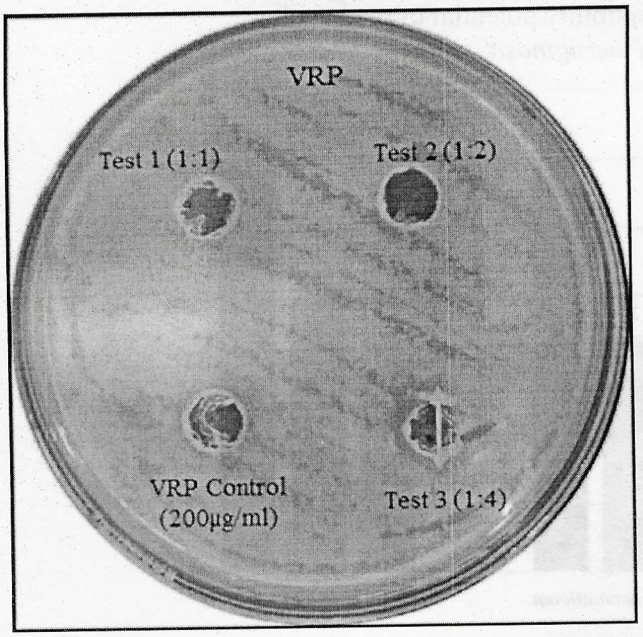

Fig. 3 Development of antibacterial susceptibility of CQ using known efflux pump inhibitor VRP. Test 1, test 2 and test 3 represent the ratio of chloroquine and verapamil co-administered against *P. aeruginosa* in 1:1, 1:2 and 1:4 ratio respectively. In control well, only VRP (200 µg/ml) was added to assess the individual inhibitory activity against *P. aeruginosa*

of VRP to enhance the antimicrobial activity of moxifloxacin and linezolid against mycobacterial infection in murine model. The study showed significant effect of VRP-mediated efflux pump inhibition that resulted in enhancement of the antimicrobial effects. Similarly, the study by Xu et al. (2017), reported that VRP enhances the activity of bedaquiline, clofazimine, and other drugs against *Mycobacterium tuberculosis*. Therefore, considering this strategy, VRP was the choice as a standard for increasing CQ susceptibility in *P. aeruginosa*.

Screening of plant methanolic extracts for modulation of CQ susceptibility against *P. aeruginosa*

The methanolic extracts of plants selected in this study were screened for development of CQ susceptibility against *P. aeruginosa* as shown in Table 1.

Out of the ten plants screened, three plants, such as *Zingiber officinale*, *Syzygium aromaticum*, *and Curcuma longa*, showed good potential of CQ susceptibility development against *P. aeruginosa* (Fig. 4).

Plants extracts- *Allium sativum*, and *Piper nigrum*, showed moderate potential which indicates that the higher concentrations of these extracts may be required for the desired activity (Table 2).

The extracts of *Syzygium aromaticum, Zingiber officinale* and *Curcuma longa* were selected for further characterization study based on their moderately good CQ susceptibility development activity against *P. aeruginosa*. These three plant extracts showed the development of zone of inhibition when administered with CQ. The diameter of zone of inhibition was observed with respect to increasing ratio of CQ: Plant extract (PE). The zone of inhibition was not observed in controls of CQ and PE in independent experiment. This indicates that the plant extract alone does not have antibacterial activity against *P. aeruginosa* at the concentration of 200 µg/ml.

Table 1 Screening of methanolic extracts of various plants for development of antibacterial susceptibility of CQ

Sr. No	Name of plant with part used in study	Potential of development of CQ susceptibility against *P. aeruginosa*
1	*Azadirachta indica (leaf)*	–
2	*Menthe longifolia (leaf)*	–
3	*Zingiber officinale (rhizome)*	++
4	*Coriandrum sativum (leaf)*	–
5	*Allium sativum (bulbs)*	+
6	*Syzygium aromaticum (bud)*	++
7	*Curcuma longa (rhizome)*	++
8	*Piper nigrum(fruit)*	+
9	*Moringa oleifera (leaf)*	–
10	*Phyllanthus emblica (fruit)*	–

Table 1 represents potential of various plant extracts for development of antibacterial susceptibility of chloroquine. '–' represents the no CQ susceptibility signified by no zone of inhibition. '+' represents the low potential of development of chloroquine susceptibility (signified by diameter of zone of inhibition < 10 mm) and '++' represents the good potential for development of chloroquine susceptibility (signified by diameter of zone of inhibition ≥ 10 mm)

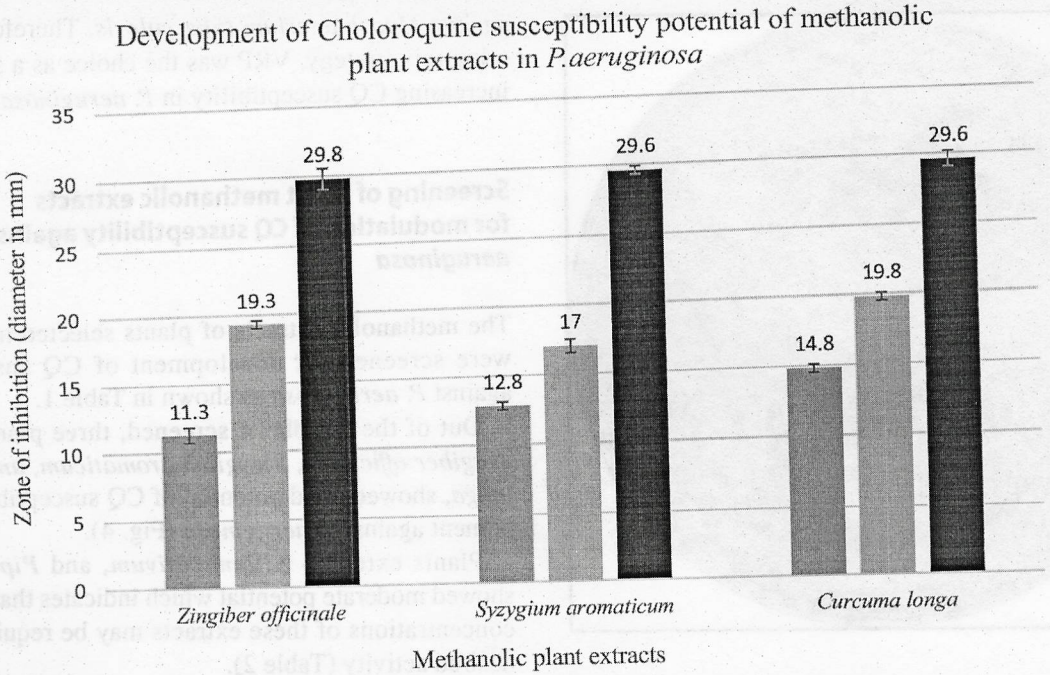

Fig. 4 CQ susceptibility development potential of three selected methanolic extracts of *Zingiber officinale*, *Syzygium aromaticum*, *and Curcuma longa* against *P.aeruginosa*

Table 2 Chloroquine susceptibility development against *P. aeruginosa* using selected plant extracts

Sr. no	Name of plant extracts	Diameter of zone of inhibition (in mm) Mean ± standard deviation				
		Control 1 CQ	Control 2 Only Plant extract (PE)	Test 1 (CQ + PE) 1:1	Test 2 (CQ + PE) 1:2	Test 3 (CQ + PE) 1:4
1	*Zingiber officinale*	NI	NI	11.3 ± 0.5	19.6 ± 0.2	29.8 ± 0.2
3	*Syzygium aromaticum*		NI	12.8 ± 0.2	17 ± 0.5	29.8 ± 0.2
4	*Curcuma longa*		NI	14.8 ± 0.2	19.8 ± 0.2	29.6 ± 0.5

Control 1 is effect of only CQ (50 µg/ml) against *P. aeruginosa*; Control 2 is effect of only plant extracts (200 µg/ml) against *P. aeruginosa* and 'NI' indicated no zone of inhibition/no susceptibility development. Test1 (50 µg/ml), Test 2 (100 µg/ml) and Test 3(200 µg/ml) represent various concentrations of plant extract along with fixed chloroquine concentration (50 µg/ml). Table 2 showed zone of inhibition of *P. aeruginosa* which is considered as susceptibility development of chloroquine

The TLC fingerprint analysis of the selected plants showed the development of several bands after iodine vapour treatment that indicates the presence various phytocomponents in each extract. Four bands were developed on TLC plate of *Syzygium aromaticum*. The R_f value calculated for individual bands was found to be 0.4,0.44,0.48 and 0.57. Similarly, five bands were developed on TLC plate of *Zingiber officinale*, with R_f value as 0.43, 0.56, 0.62, 0.75 and 0.81. In case of *Curcuma longa*, four bands with R_f value as 0.37, 0.42, 0.54 and 0.62 were developed on TLC plate. All the bands were isolated and were further evaluated for CQ susceptibility development against *P. aeruginosa*.

Assessment of phytocomponents isolated from TLC bands for development of CQ susceptibility against *P. aeruginosa*

All the developed bands on TLC were isolated and evaluated for development of CQ susceptibility in *P. aeruginosa*. In the case of *Syzygium aromaticum,* out of four bands evaluated, only band with R_f value 0.44 showed the formation of zone of inhibition when combined with CQ. Similarly, in case of *Zingiber officinale* out of five bands evaluated, two bands with R_f value 0.43 and 0.75 showed antibacterial activity in combination with CQ. In case of *Curcuma longa,* two bands with R_f value 0.54 and 0.62 showed antibacterial activity in combination with CQ. (Fig. 6).

The zone of inhibition was observed when the isolated phytochemicals were combined with CQ illustrated in Table 4. The controls of CQ and phytocomponents at 50 µg/ml (a maximum concentration used in this assay) individually did not show any antibacterial activity against *P. aeruginosa*.

Phytochemical profiling of isolated phytocomponents

Towards the identification of active principle of selected plant extracts for drug sensitivity enhancement, thin-layer chromatography was performed and various phytocomponent bands were isolated. The active bands were further analysed for phytochemical profiling (Prabhavathi et al. 2016; Shah and Seth 2010). Table 5 illustrates the findings of phytochemical analysis of isolated active bands.

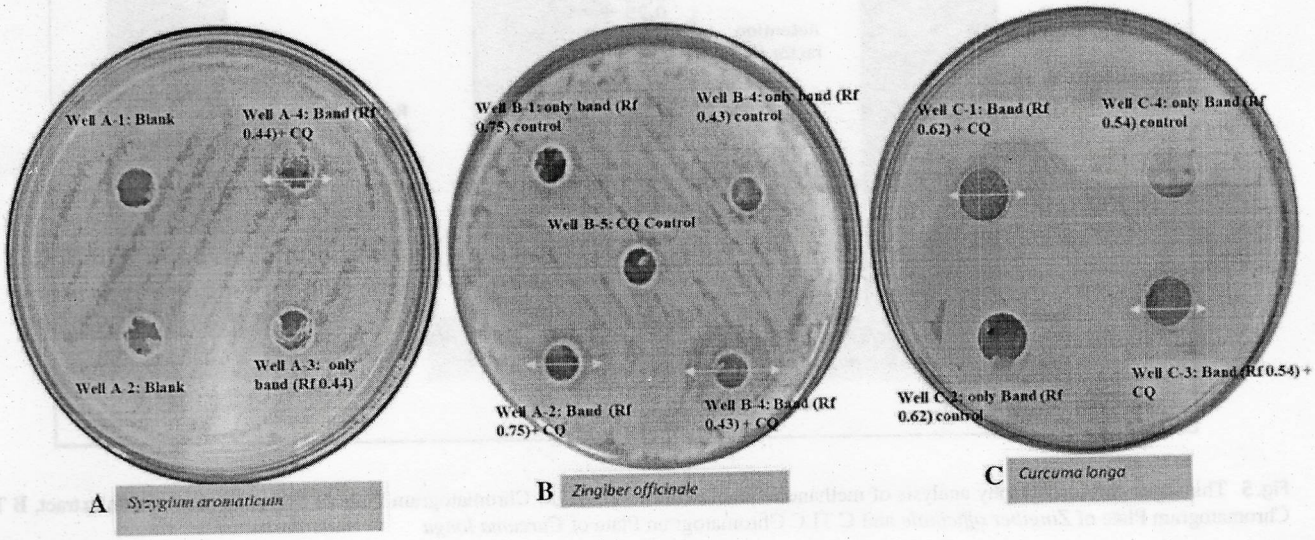

Fig. 6 CQ susceptibility of isolated phytochemicals against *P. aeruginosa*

Table 4 Assessment of CQ susceptibility of the phytocomponents isolated from methanolic extracts of plants against *P. aeruginosa*

Sr. no	Plant methanolic extracts	Rf value of active band	Diameter of Zone of inhibition (Mean ± Standard deviation)
1	*Syzygium aromaticum*	0.44	13±0.05
2	*Zingiber officinale*	0.43	26±0.15
		0.75	12±0.05
3	*Curcuma longa*	0.54	11±0.05
		0.62	09±0.11

Table 4 represent the potential of the active bands isolated from the selected methanolic plant extracts namely, *Syzygium aromaticum*, *Zingiber officinale* and *Curcuma longa* for the development of chloroquine susceptibility against *P. aeruginosa* measured as zone of inhibition

Thin-layer chromatographic analysis of selected plant extracts

The three best methanolic plant extracts namely, *Syzygium aromaticum, Zingiber officinale, and Curcuma longa* were selected for TLC analysis to isolate the active phytocomponent responsible for the development of antibacterial susceptibility of CQ against *P. aeruginosa*. Figure 5 showed the TLC chromatogram of selected plant extracts.

Three selected plant extracts were further qualitatively analysed for the possible presence of phytochemicals by various biochemical methods (Table 3).

Various phytochemicals, such as quinones, phenol, triterpenoid, saponins, tannins, alkaloids and flavonoids, were found in the *Syzygium aromaticum* extract. The presence of saponins, tannins, alkaloids and flavonoids were found in methanolic extract of *Zingiber officinale* and *Curcuma longa*. Similar phytochemical analytical work was done by Ali et al. (2018) who reported the presence of alkaloids, flavonoid, glycosides, reducing sugar, saponin, steroids, phenols, terpenoid, anthraquinones in methanolic extract of *Syzygium aromaticum*. Likewise, a study by Bashir et al. (2015) reported presence of tannins, alkaloids and flavonoids in methanolic extract of *Zingiber officinale*. Similarly, carbohydrate, proteins, starch, amino acids, steroid, glycoside saponins, tannins, alkaloids, flavonoids are reported to be present in methanolic extract of *Curcuma longa*.

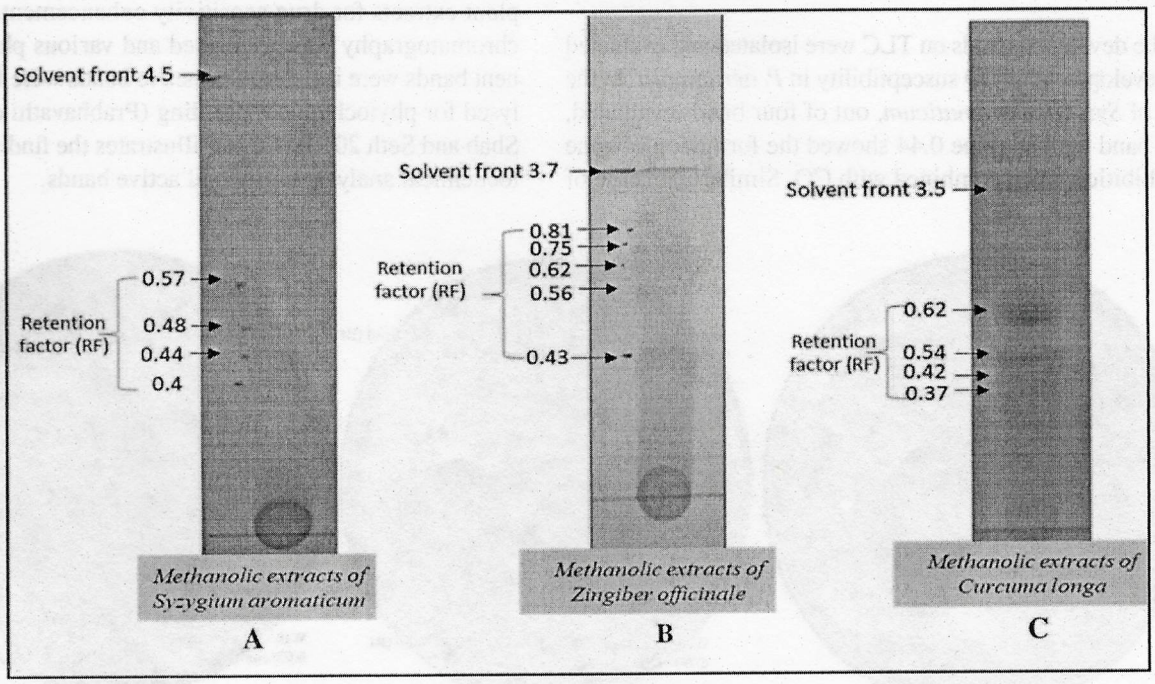

Fig. 5 Thin-layer chromatography analysis of methanolic plant extracts of **A** TLC Chromatogram Plate of *Syzygium* aromaticum extract, **B** TLC Chromatogram Plate of *Zingiber officinale* and **C** TLC Chromatogram Plate of *Curcuma longa*

Table 3 Phytochemical (qualitative) analysis of selected methanolic extract of plants

Plant methanolic extract	Phytochemicals						
	Quinones	Saponin	Tannin	Alkaloid	Phenols	Tri-terpenoids	Flavonoids
Syzygium aromaticum	+	+	+	+	+	+	+
Zingiber officinale	–	+	+	+	–	–	+
Curcuma longa	–	+	+	+	+	–	+

Table 3 represents the presence of various phytochemicals in the selected plant extracts identified by biochemical tests. The *Syzygium aromaticum* extract showed the presence of all the phytochemicals tested. On the other hand, the methanolic extract of *Zingiber officinale* showed the presence of saponins, tannins, Alkaloids and flavonoids. In case of *Curcuma longa* extract of saponins, tannins, alkaloids, phenols and flavonoids were present

Indications: '–' Absence of phytochemical; "+": Presence of Phytochemical

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Table 5 Phytochemical analysis of active principle isolated from TLC bands

Active bands isolated from Plant methanolic extracts	Phytochemicals						
	Quinones	Saponin	Tannin	Alkaloid	Phenols	Tri-terpe-noids	Flavonoids
Band (Rf 0.44) from *Syzygium aromaticum extract*	–	–	–	–	–	–	+
Band (Rf 0.43) from *Zingiber officinale extract*	–	–	–	+	–	–	–
Band (Rf 0.62) from *Curcuma longa extract*	–	–	–	–	+	–	–

Table 5 represents the qualitative phytochemical profiling of the active band isolated from the selected methanolic plant extracts. The band (Rf 0.44) isolated from *Syzygium aromaticum* extracts showed the presence of only flavonoids. The presence of only alkaloids was shown by band (0.44) isolated from *Zingiber officinale*. The band (Rf 0.62) isolated from *Curcuma longa* extracts showed the presence of only phenols

Indications: '–' Absence of phytochemical; "+": Presence of Phytochemical

The findings of the phytochemical analysis indicates that, flavonoid (R_f 0.44) in *Syzygium aromaticum,* alkaloid (R_f 0.43) in *Zingiber officinale* and phenol (R_f 0.62) in *Curcuma longa* were found responsible for enhancement of CQ susceptibility in *P. aeruginosa.*

In case of the remaining screened phytocomponent bands from *Zingiber officinale* with R_f 0.75 and *Curcuma longa* with R_f 0.54, the phytochemical profile was not clearly obtained. Although a drug resistant strain of *P. aeruginosa* was not used in the present work, further studies are required in this direction for identification and structural characterization of active phytocomponents responsible for the development CQ susceptibility.

Conclusion

Present investigation successfully demonstrated the attractive concept for the enhancement of chloroquine sensitivity in bacterial system by modulating an efflux pump. Study explored the potential of plants for development of antibacterial susceptibility of CQ in *P. aeruginosa.* Plant extracts and isolated phytochemicals have shown good candidature for increasing susceptibility of CQ in *P. aeruginosa.* Present investigation broadly revealed that the phytochemicals viz. a selected flavonoid from *Syzygium aromaticum, an* alkaloid from *Zingiber officinale* and a phenol from *Curcuma longa* are important components for drug sensitivity enhancement. Therefore, these phytocomponents can be explored for drug sensitivity enhancement or even for reversal of drug resistance. In this investigation, indirectly efflux pumps were targeted using VRP-based assay as standard. Study provides a very simple strategy or outline for CQ sensitivity enhancement in bacterial system using an efflux pump inhibitor can be used either for development or enhancement of drug sensitivity. The concept can be explored for repurposing chloroquine as effective antibacterial agent in the presence of plants and their phytochemicals.

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Author contributions All the authors participated in discussion during manuscript planning and writing.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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